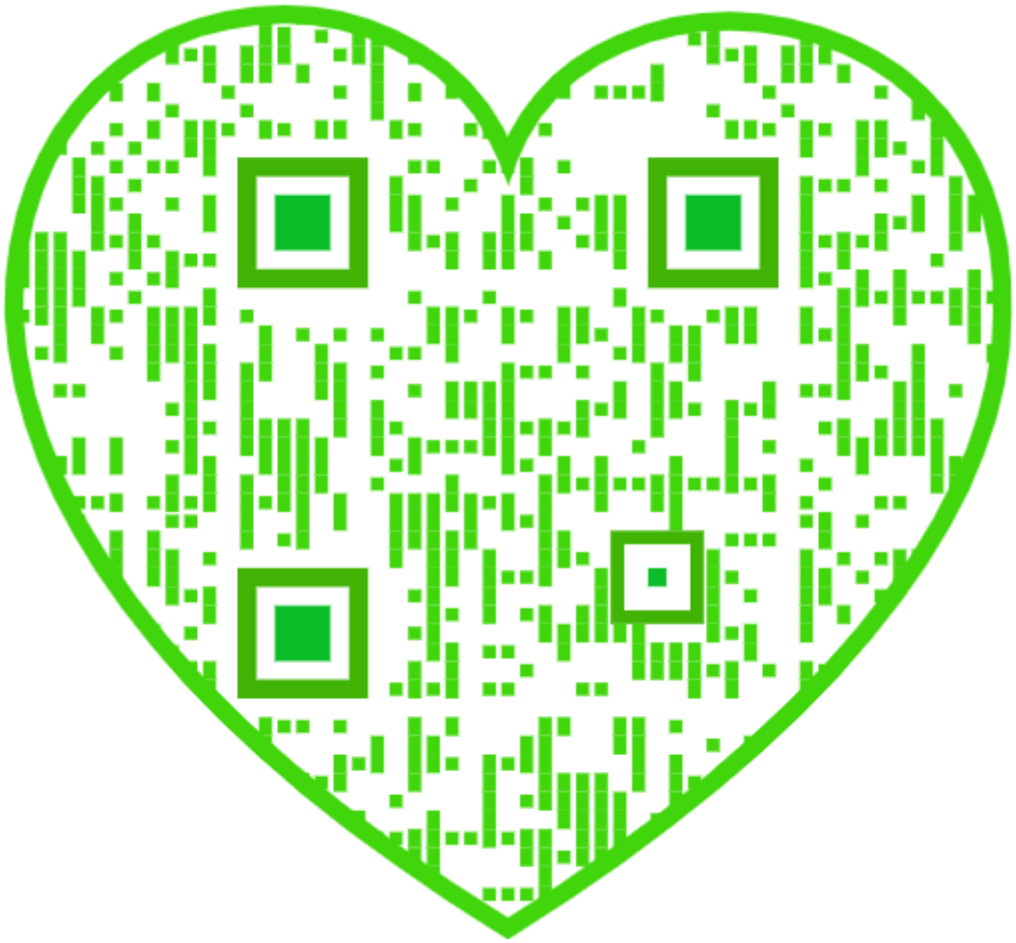
UNIVERSITY OF TORONTO
3 1761 01288798 0



Digitized by the Internet Archive
in 2007 with funding from
Microsoft Corporation

http://www.archive.org/details/laboratorystudie00browuoft

LABORATORY STUDIES

FOR

BREWING STUDENTS

FERMENTATION ORGANISMS:

A Laboratory Handbook.

By ALB. KLÖCKER.

Translated by G. E. Allan, B.Sc., and J. H. Millar, F.I.C.

With 146 Illustrations in the Text. 8vo, 12*s.* net.

THE CHEMICAL CHANGES AND PRODUCTS RESULTING FROM FERMENTATIONS.

By R. H. ADERS PLIMMER.

8vo, 6*s.* net.

LONGMANS, GREEN, AND CO.,

39 Paternoster Row, London, E.C.

TC
Bro

LABORATORY STUDIES

FOR

BREWING STUDENTS

A SYSTEMATIC COURSE OF PRACTICAL WORK IN THE SCIENTIFIC PRINCIPLES UNDERLYING THE PROCESSES OF MALTING AND BREWING

BY

ADRIAN J. BROWN, M.Sc.

DIRECTOR OF THE SCHOOL OF BREWING, AND PROFESSOR OF THE BIOLOGY AND CHEMISTRY OF FERMENTATION IN THE UNIVERSITY OF BIRMINGHAM
EXAMINER IN BIOLOGICAL CHEMISTRY TO THE INSTITUTE OF CHEMISTRY, ETC.

162300.
28.S.21.

WITH 36 ILLUSTRATIONS

LONGMANS, GREEN, AND CO.
39 PATERNOSTER ROW, LONDON
NEW YORK AND BOMBAY
1904

PREFACE.

Some years ago, when it fell to the author's lot to arrange a course of instruction in the principles of brewing for his students at the University of Birmingham, an examination of the literature of the subject showed that there was no book in existence which could be used as a systematic guide to practical work in the laboratory, and as the author recognised that a sound knowledge of the principles of brewing must be based on work of this nature, it became necessary to draw up a course of laboratory studies for the special use of his students. This course, subject to alterations and additions suggested by experience and by the progress of knowledge, has now been in use for several years, and as it has been found to fulfil its requirements in a satisfactory manner, the author now ventures to publish it in the hope that it may contribute in some measure towards filling a gap in the literature of brewing.

The work is essentially a student's laboratory

guide, and must not be regarded in any way as a text-book of the scientific principles of brewing as it confines itself mainly to descriptions of experimental work. It is intended for use under the supervision of a competent instructor, and it is assumed that the student is able to attend lectures on the subjects upon which he is working.

The chief difficulty of a study of the scientific principles underlying brewing practice lies in the fact that as it so often touches the limits of our present knowledge there are many questions which have to be studied about which there exists considerable uncertainty and difference of opinion. This naturally raises difficulties for the teacher, for, on the one hand, it is well recognised that some amount of dogmatism in teaching is necessary when introducing a new subject to the student, and, on the other, the state of our knowledge of certain of the questions dealt with in these studies does not justify dogmatic treatment. The position may be illustrated by a consideration of the very important and difficult problems of the constitution of the starch molecule and its transformation by diastase. A large amount of knowledge on these points has been accumulated, and many varied views have been advanced by different investigators concerning them, but none of these views have met with general acceptance, even of a provisional

nature. This is due no doubt partly to the exceptional difficulties which surround the study of starch and its transformation by diastase, and partly to a militant spirit which appears to emanate from the starch molecule and influence the minds of most of its investigators.

How is this state of affairs to be met by the teacher? An attempt to lay before the student at the commencement of his studies all the different views concerning starch and its transformation products must result in reducing his mind to a state of chaos. It appears to the author that the only satisfactory course open is to teach those views which lend themselves best to explanation and demonstration, and, when the student is sufficiently advanced, to encourage him to criticise such views and compare them with others.

This is the method of teaching attempted by the author, and it is followed in this book with regard both to experimental studies, and to a consideration of the analytical processes employed in the brewery laboratory, many of which cannot be regarded as above criticism.

The course of studies might perhaps have been advantageously lengthened, but it had to be borne in mind that the majority of students are unable to devote more than one year to such studies. A good worker who has previously had a sound

chemical training is able to work through the whole course in this time; but, for the benefit of those who have had a less complete preliminary training or who are less expert workers, some of the experiments are printed in small type to indicate that they may be omitted.

The references given in this book to original papers are not intended to be exhaustive, but have been selected in order to encourage the student to refer to original sources for information. In making the selection regard has been paid to those sources which are likely to be readily available.

The author desires to express his indebtedness to Mr. J. H. Millar for very valuable assistance in planning the studies of the carbo-hydrates, and also to Mr. Thomas H. Pope for kindly reading over the proof-sheets of this work, and for giving many useful suggestions.

School of Brewing,
University of Birmingham,
June, 1904.

CONTENTS.

PAGE

PREFACE v

SECTION I.—BARLEY AND MALTING.

PART I. A STUDY OF THE BARLEY CORN 1

The General Characteristics of a Grain of Barley 1
Ear of Ripe Barley and Spike of Barley when in Flower compared 3
The Flower of Barley 3
Ovary 4
Anthers 5
Lodicules 5
The Flower after Fertilisation 6
Barley and Wheat compared 6
Ears of Two-rowed and Six-rowed Barley compared . . . 7
Characteristics of Sub-species of Barley 9
Ears of Chevalier and Goldthorpe Barley compared . . . 10
Basal Bristles of Chevalier and Goldthorpe Barley . . . 11
Corns of Chevalier and Goldthorpe Barley compared . . . 11
Transverse Furrow of Goldthorpe Barley 11
Hordeum vulgare and *H. hexastichum* compared 12

PART II. ANATOMY OF THE BARLEY CORN 13

Endosperm and Embryo of the Barley Corn 13
Cutting and Preparing Sections for the Microscope . . . 14
Anatomy of the Embryo 16
Anatomy of the Endosperm 17
The True Skins of the Barley Corn 19
Examination of Green Six-rowed Barley 19
Position of Starch Granules in the Cells of the Endosperm . 20
Demonstration that the Starch-containing Cells contain Protoplasmic Matter 20

PAGE

PART III. EXPERIMENTS CONNECTED WITH THE TECHNICAL STUDY OF BARLEY AND OTHER CEREALS 22

Microscopic Appearance of the Commoner Kinds of Starch . 22
Preparation of Starch from Barley 22
Determination of Moisture in Barley 23
Determination of the "Vitality" or Germinative Power of Barley 24
The "Weight" of Barley 24
Specific Gravity of Barley 25
Technical Examination and Valuation of Barley for Malting Purposes 26

PART IV. THE CHANGES IN BARLEY DURING GERMINATION . . 27

Determination of Moisture in Steeped Barley 27
Study of the Changes in Barley during Germination . . . 27
Growth of the Embryo when Separated from its Endosperm . 30
The Embryo of the Barley Corn secretes Diastase during its Development 31
Generation of Carbon Dioxide during the Growth of the Barley Corn 33
Oxygen is Absorbed during Growth 34
Preparation of Malt Diastase 34
Action of the Enzyme Cytase 35
Loss in Weight during the Conversion of Barley into Malt . . 36
Technical Examination of Malt 37
1. General Appearance 37
2. "Modification" 37
3. "Condition" 37
4. Flavour 37
5. Regularity of Growth 37
6. Damaged Corns 38
7. Mouldy Corns 38
The "Sinker" Test 38
Specific Gravity of Malt 38

PART V. THE CHEMICAL ANALYSIS OF MALT 39

Moisture in Malt 39
Ash 39
Determination of the Extract of Malt 39
Determination of the Specific Gravity of Liquids . . . 40
Preparation of the Malt Mash (Heron's Method) . . . 42
Extract of the Boiled Wort 44
Calculation of Dry Extract 44
Calculation of Weight of Dry "Grains" 45
Influence of Grinding on Extract 46
Criticism of the Method Employed 46

PAGE

Determination of Extract by Weighing the Mash . . . 47
The "Full Theoretical" Extract 49
Extract of "Flaked" and Prepared Grain 50
Extract of Black and Brown Malt 51
Extract of Raw Grain 51
Acidity of Malt 52
Determination of the Diastatic Power of Malt (Lintner's Method) 53
Diastatic Power of Barley 55
"Non-coagulable Albuminoids" of Malt 55
"Ready-formed Carbo-hydrates" of Malt 59
Soluble Ash and Colour of Malt 61
"Saccharification" Test 62

SECTION II.—Principles of the Mashing Process.

Part I. A Course of Experiments Constituting a Study of Some of the Carbo-hydrates Concerned in Wort Production, and Introducing the Student to the Special Methods Employed in their Examination 64

Action of Water on Starch 64
Preparation of Soluble Starch 65
Hydrolysis of Starch by Acid 65
Preparation of Dextrose 67
Quantitative Study of the Hydrolysis of Starch by Acid . . 68
Determination and Use of the "Solution Weights" of Carbo-hydrates 69
Solution Density of Dextrose 71
Introduction to the Use of the Polarimeter in Carbo-hydrate Work 72
The Specific Rotation of Dextrose determined by means of the Sodium-light Polarimeter 75
The Specific Rotation of Dextrose determined by means of the Half-shadow Polarimeter 75
Use of a Factor other than the True Solution Factor in Determinations of the Specific Rotations of Carbo-hydrates . 76
Determination with the Polarimeter of the Amount of Sugar present in a Solution when the $[a]_D$ of the Dissolved Sugar is known 76
"Mutarotation" of Dextrose and other Carbo-hydrates . . 77
The Cupric Oxide Reducing Method of Estimating Sugars . . 78
Preparation of Fehling's Solution 78
Determination of the Reducing Power of Dextrose . . 79
Preparation and Properties of Phenyl-glucosazone . . . 81

PAGE

Cane-Sugar or Saccharose 82
Cane-Sugar does not possess Cupric Oxide Reducing Power 82
Cane-Sugar does not Combine with Phenyl-hydrazine to form an Osazone 82
Inversion of Cane-Sugar 82
Inversion with Acid 83
Inversion with Yeast 83
Determination of the Cane-Sugar present in Malt . . . 85
Levulose 86
Specific Rotation 86
Reducing Power 86
Preparation of Osazone 86

PART II. STUDY OF THE HYDROLYSIS OF STARCH BY DIASTASE, AND OF THE PRODUCTS OF HYDROLYSIS 87

Preparation of Cold-water Malt Extract 87
Action of Diastase on Starch 87
Action of Diastase is Destroyed at 100° 88
Preparation of Maltose 88
Specific Rotation of Maltose 90
Reducing Power of Maltose 91
Preparation and Properties of Phenyl-maltosazone . . . 91
Hydrolysis of Maltose by Acid 92
Maltose is not Hydrolysed by the Action of Invertase . . . 92
Dextrin 93
Preparation 93
Specific Rotation 93
Reducing Power 93
Preparation and Quantitative Examination of a Low Starch Conversion 94
Influence of Heat on the Hydrolysis of Starch by Diastase . . . 98
Influence of Heat in Restricting Starch Transformation is Due to Modification of the Diastase 99
Experiment Showing that Although a Restricted Starch Transformation may be Represented as a Mixture of Maltose and Dextrin, much of the apparent Maltose cannot be Fermented by Ordinary Yeast and Exists as Malto-dextrin 99
Preparation of the Starch Conversion 100
Determination of the Free Maltose Present 101
Calculation of the "Apparent" Maltose present which exists as Malto-dextrin 102
Determination of the "Apparent" Dextrin existing as Malto-dextrin 102
Constitution of the Malto-dextrin found 104
Determination of the Stable Dextrin 104

PAGE

PART III. STUDIES BEARING DIRECTLY ON THE TECHNOLOGY OF BREWING 106

Study of the Influence of the Mashing Temperature on the Optical Activity of Malt Worts 106

Experiments on the Fermentation of Boiled and Unboiled Worts Illustrating the Different Methods of the Brewer and the Distiller or Vinegar Maker 107

Study of the Rise in Temperature observed when mixing Dry Malt with Cold Water 108

Analysis of a Fermented Wort or Beer 110

1. Determination of the "Original Gravity" and Alcohol . 110

2. Analysis of the Unfermented Matter 114

Free Maltose 114

"Apparent" Maltose Combined as Malto-dextrin . . 115

"Apparent" Dextrin Combined as Malto-dextrin . . 115

Stable Dextrin 116

Residual Reducing and Rotatory Powers . . . 116

PART IV. ANALYSIS OF BREWING SUGARS 117

Analysis of Invert-Sugars 117

Ash 117

Albuminoids 118

Brewer's Extract and Water 118

Reducing Sugars 119

Cane-Sugar 120

Unfermentable Matter 121

Example 122

Analysis of Glucose or Starch Sugar 125

SECTION III.—FERMENTATION.

INTRODUCTION 126

PART I. THE PHYSIOLOGICAL ASPECT OF FERMENTATION . . . 127

Determination of the Amount of Alcohol and Carbon Dioxide produced during the Fermentation of Sugar by Yeast . 127

Glycerin a Secondary Product of Alcoholic Fermentation . . 130

Chemical Composition of Yeast 131

Water 131

Ash 132

Nitrogen 132

Use of the Hæmacytometer 133

Nature of the Food Requirements of the Yeast Cell . . . 137

PAGE

Multiplication of Yeast Cells in a Nutritive Solution not Directly Dependent on the Number of Cells Originally Introduced 138
Multiplication of Yeast Cells in a Nutritive Solution not Directly Dependent on the Amount of Yeast Food Present . . 139
Removal of Nitrogen from Malt Wort during Fermentation . 140
Influence of Temperature on the Development and Fermentative Power of Yeast 140
Actions of some of the Enzymes Present in the Yeast Cell . . 142
1. Zymase 142
2. Invertase 142
3. Maltase 142
Auto-digestion of Yeast. 143

PART II. A STUDY OF THE MORPHOLOGY AND LIFE HISTORY OF SOME OF THE MORE IMPORTANT MICRO-ORGANISMS OF FERMENTATION WHICH INTRODUCES THE STUDENT TO THE SPECIAL METHODS EMPLOYED IN THE STUDY OF THE FERMENTATION ORGANISMS 144

Preparation of Culture Media 145
Hopped Malt Wort 145
Yeast Water 145
Meat Extract 146
Wort Gelatin 146
Preparation of Flasks and Test-Tubes Containing Malt Wort and Gelatin Malt Wort 147
Sterile Water. 148
A. The Saccharomycetes and Lower Fungi—
Study of the Morphology of a Yeast Cell 148
Growth of Yeast in a "Drop Culture" 151
Study of Some of the Well-recognised Races of Yeasts and Torula 153
Study of Some of the more Common Species of Moulds . 154
Preparation of Spore Cultures of the Saccharomycetes . 155
Film Formation of the Saccharomycetes 158
Preparation of Pure Cultures of Yeasts and Moulds . . 159
Gelatin Plate Method 159
Hansen's Single Cell Method 160
Analysis of Mixed Cultures of Yeast 162
Gelatin Plate Method 163
Fractional Culture Method 164
Method of Testing Pure Cultures of Yeasts obtained by Previous Methods of Analysis for Technical Purposes 165
Experiments on the Powers of Hydrolysis and Fermentation of Different Species of Yeasts 166

PAGE

B. The Schizomycetes or Bacteria—
Morphology of a Bacterium (Bacillus subtilis) . . . 166
Making Stained Preparations 168
Spore Formation 169
Preparation of a Drop Culture of Spores . . . 169
Study of Some of the Well-recognised Species of Acid-producing Bacteria 169
Examination of Beer Sediments 170
The "Forcing" Test 171
Micro-organisms Present on Barley, Malt and Hops . . 171
Biological Examination of Air for Technical Purposes . . 172
Roll Culture Method 172
Petri Dish Method 173
Biological Examination of Water for Technical Purposes . 173
Biological Examination of Water for Hygienic Purposes . 174

SECTION IV.—The Hop.

The Hop Plant 176
The Female Flower of the Hop 176
The Hop-Cone 178
Bracts of the Hop-Cone 179
The Stem or "Strig" of the Hop 179
Lupulin Glands of the Hop 181
Comparison of the Strig of Different Varieties of the Hop . . . 182
Chemical Examination of Hops 183
The Soft and Hard Resins 183
Moisture 185
Detection of Sulphur 185
Tannic Acid 186

Table I. Solution Factors for Carbo-hydrates at Various Densities . 187

Table II. Divisors for the Transformation Products of the Hydrolysis of Starch 188

Table III. Reducing Values of Varying Quantities of Dextrose, Levulose and Invert-Sugar 189

Table IV. Reducing Values of Varying Quantities of Maltose . . 191

Table V. Spirit Indication Table 192

Table VI. Table for Ascertaining the Correction for Acid in Original Gravity Determinations 193

ILLUSTRATIONS.

FIG.

1. Barley Flower . . . . . *page* 4
2. Lodicules of Barley Flower . . . . . *facing page* 4
3. Ovary of Barley Flower . . . . . *page* 4
4. Diagram of a Transverse Section through the Ovary of a Barley Flower . . . . . „ 5
5. Photomicrograph of Anthers and Lodicules from an Undeveloped Barley Flower . . . *facing page* 6
6. Photomicrograph of Transverse Section of a Fertilised and Developing Ovary . . . . . „ 6
7. (*a*) Six-rowed Barley; (*b*) Two-rowed Barley . . . *page* 7
8. (*a*) *Hordeum distichum*; (*b*) *Hordeum zeocriton* . . . „ 10
9. (*a*) Rachis of Goldthorpe Barley; (*b*) Rachis of Chevalier Barley . . . . . „ 10
10. (*a*) Basal Bristle of Goldthorpe Barley; (*b*) Basal Bristle of Chevalier Barley . . . . . *facing page* 11
11. *Hordeum vulgare* . . . . . *page* 12
12. *Hordeum hexastichum* . . . . . „ 12
13. Diagram of a Longitudinal Section through the Germ End of a Barley Corn . . . . . „ 15
14. Diagram of a Transverse Section through a Barley Corn . „ 18
15. Photomicrograph of Transverse Section through a Barley Corn, showing the Funicle . . *facing page* 19
16. Photomicrograph of Section of Skins and Endosperm of Barley Corn (highly magnified) . . „ 19
17. Experiment Demonstrating the Secretion of Diastase by the Growing Embryo of a Barley Corn . . . . . *page* 32
18. Apparatus for Demonstrating that Carbon Dioxide is Generated during the Respiration of Germinating Barley . „ 33
19. Apparatus for the Determination of Carbon Dioxide and Alcohol Generated during Alcoholic Fermentation . . „ 128
20. Figure of Thoma's Hæmacytometer . . . . . „ 133
21. Freudenreich Flask . . . . . „ 147
22. Holder for Sterile Water . . . . . „ 147

FIG.
23. Moist Chamber . . . . . *page* 151
24. Multiplication of Top Yeast . . . . . „ 153
25. Gypsum Block . . . . . „ 156
26. Sporulation of *Saccharomyces Cerevisiæ* . . . . . „ 157
27. Saccharomycetes which form Ascospores . . . . . „ 157
28. Squared Cover-glass . . . . . „ 161
29. Object Marker . . . . . „ 161
30. *Clostridium butyricum* . . . . . „ 167
31. Female Inflorescence of the Hop . . . . . „ 177
32. Diagram of a Female Flower of the Hop . . . . . „ 177
33. Bracts of Hop-cone (*a*) Stipular Bract; (*b*) Bracteole . . „ 178
34. Axis or "Strig" of Hop-cone . . . . . „ 179
35. Group of Floral Axes on Hop Strig (much enlarged) . . „ 179
36. Apparatus for Extraction of Hop Resins . . . . . „ 183

LABORATORY STUDIES
FOR
BREWING STUDENTS.

SECTION I.

BARLEY AND MALTING.

PART I.

A STUDY OF THE BARLEY CORN.

Examine a Grain of ordinary English Chevalier Barley.—Note that the grain is spindle-shaped or fusiform, and is about one-third of an inch in length. On closer inspection it will be observed that one end of the grain, which is somewhat sharper than the other, shows signs of having been fractured. This is the end which was originally attached to the ear of barley previous to threshing, and is consequently the lower end of the grain.

Observe that a deep, narrow furrow runs down the more convex side of the grain. This furrow is termed the *ventral furrow*, and the side of the grain on which it is situated is termed the *ventral side.*

The other side—which is flatter than the ventral side—is termed the *dorsal side.*

Examine the ventral furrow towards the lower end of the grain, and notice that within it there lies a thin bristle. Remove the bristle with the point of a dissecting needle, and examine it under the microscope with a low-power (1 inch) lens. It will be found that it is covered with fine lateral hairs. When removing the bristle, usually termed the *basal bristle,* from the ventral furrow, notice that it is attached to the base of the grain close to the point where the grain was originally connected with the ear. The basal bristle, or rachilla, is merely the prolongation of the axis or point from which the corn was originally developed. It has no physiological importance, but its appearance is sometimes useful in assisting to discriminate between different kinds of barley.

Examine a Grain of Barley when softened by soaking in Cold Water for about forty-eight hours. —Observe that it is covered by a thick skin. A careful examination will show that this skin is not continuous round the grain, but consists of two separate portions, one closely adhering to the dorsal and the other to the ventral side of the corn, and that the skin on the dorsal side overlaps the edges of the skin covering the ventral side. With care the two skins may be removed entire, and it will then be noticed how much their general structure resembles that of a grass leaf—the dorsal skin

especially showing conspicuous longitudinal veins, or vascular bundles, similar to those in a grass leaf.

Compare the Barley under Examination with an Ear of Unthreshed Barley.—It will be noticed that the furrowed or ventral side of a grain of barley is the side which is turned inward towards the stem, or rachis. It will also be noticed that the *awn*, or *beard*, of unthreshed barley is merely a prolongation of the skin which covers the dorsal side of the barley corn, and that it is broken off during the threshing process.

Compare an Ear of Ripe Barley with a Spike of Barley when in Flower.—A general similarity between the two is apparent. The flowers are arranged alternately on the stem or rachis, and the outer covering of the flower with its long awn at once suggests that this covering is the same as the thick skin of the barley corn.

Detach a Flower from the Spike.—Notice that the outer covering of the flower is composed of the same two leaf-like structures which were found enclosing the barley corn. The one which partially wraps round the other and is terminated by the awn, is called the *palea inferior*, and the other the *palea superior*.

Open the Flower and Remove the Palea Inferior.—The organs of the flower will then be observed lying within the fold of the *palea superior*. These consist of :—

(*a*) An *ovary*. (The female organ.)

(*b*) Three pollen-bearing *anthers*, each supported on a hair-like filament. (The male organs.)

(*c*) Two minute transparent leaflets called *lodicules* which are situated at the base of the ovary and embrace it. (The lodicules are sometimes regarded as abortive petals of the flower.)

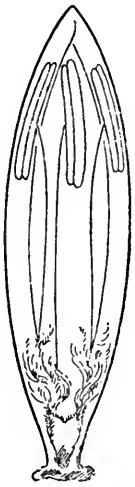


FIG. 1.—Barley Flower with Palea Inferior removed exposing the Ovary and Anthers.

FIG. 3.—Ovary of Barley Flower.

Remove the Ovary with a Dissecting Needle and Examine it under a low Microscopic Power.—It is roughly heart-shaped and is surmounted by two hairy *stigmata* adapted for the purpose of retaining pollen grains. On the inner side of the ovary (the side nearest to the rachis), a furrow will be seen which is retained during development into a barley corn and thus represents the ventral furrow.

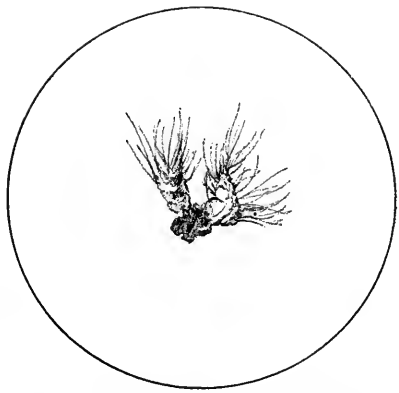


Fig. 2.—Lodicules of Barley Flower.

A practical study of the structure of the ovary is too difficult for the ordinary student to attempt, as it is not easy to cut sections of it which are suitable for microscopical examination. The ovary is mainly composed of soft, thin-walled cells, or parenchyma, which enclose a single *ovule* surrounded by double walls or integuments (see Fig. 4). The interior of the ovule is occupied by a very large cell called the *embryo-sac*. Note that the ovule lies free within the ovary except at one point called the *funicle*, where it is attached to the inner walls of the ovary. The thick walls of the ovary are almost colourless with the

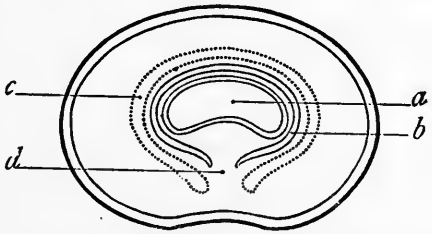


FIG. 4.—Diagram of a Transverse Section through the Ovary of a Barley Flower. (*a*) Embryo-sac; (*b*) Integuments of Ovule; (*c*) Layer of Cells Containing Chlorophyll; (*d*) Point of Attachment of Ovule.

exception of one layer of cells coloured bright green by chlorophyll; this layer may be easily seen in a transverse section of a fresh ovary.

Remove an Anther from the Flower.—Mount it on a slide with water and cover with a thin glass. Observe under the microscope the pollen grains contained within the anther.

Dissect out the Lodicules at the Base of the Ovary.—Note their exact position within the paleæ with a view to tracing them subsequently in the ripe barley corn.

Mount the Lodicules in Water.—Examine them under the microscope, noting their structure and

the thin transparent hairs with which they are covered.

Again Examine the Spike of Barley in Flower.—Observe that on either side of a fully developed flower, and springing from the same internode, is an undeveloped flower sheath about one-third of an inch in length. Close examination will show that this sheath consists of two incompletely developed paleæ. They enclose merely an abortive trace of an ovary, but occasionally fully developed anthers and lodicules are found within the flower sheath.

Examine a Spike of Barley collected a few days after Fertilisation.—Note how rapidly the ovaries are increasing in size and tending to fill the space enclosed by the flower sheaths.

Fertilisation of the barley flower is effected when pollen grains shed by the anthers come into contact with the stigmata of the ovary. A minute tube carrying a nucleus is extended from a pollen grain into the conducting tissue of the ovary, and after finding its way through the micropyle of the ovule to the embryo-sac, pierces it, leading to fusion of the nuclei of the embryo-sac and pollen grain. After fertilisation has been effected in this manner, the cells within the embryo-sac commence to divide and subdivide, eventually producing the embryo and endosperm of the barley corn to be referred to later on. Fig. 6 is a micro-photograph of a fertilised and developing ovary; note the enlargement of the embryo-sac and the compression of the surrounding walls of the ovule and ovary. Eventually these walls become the thin skins of the barley corn.

Compare a Spike of Ripe Two-rowed Barley

FIG. 5.—Photomicrograph of Anthers and Lodicules from an Undeveloped Barley Flower.

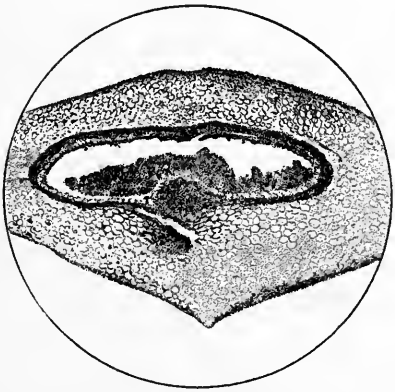


FIG. 6.—Photomicrograph of Transverse Section of a Fertilised and Developing Ovary.

with a Spike of Ripe Wheat.—Observe in the case of barley that the ovary has increased in size until it has completely filled the space enclosed by the paleæ, and that the latter adhere firmly to the grain, forming the outer covering or false skin of the grain. In the case of wheat, note that the paleæ enclosing the grain do not adhere, and that the seed is therefore naked after threshing, the paleæ being separated as chaff.

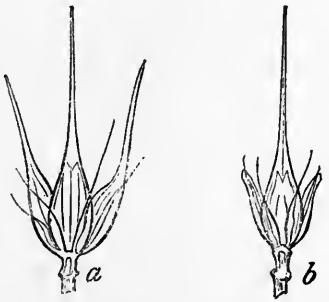


FIG. 7.—(*a*) Six-rowed Barley; (*b*) Two-rowed Barley.

Compare a Spike of Ripe Six-rowed Barley (Hordeum vulgare) with a Spike of Two-rowed Chevalier Barley (H. distichum).—In the two-rowed barley it will be noticed that on either side of each corn there is an undeveloped flower, but in the six-rowed barley all the flowers have developed into corns, thus making three rows of corns on each side of the spike. The difference between six-rowed and two-rowed barley consists therefore in all three flowers of the six-rowed barley being fully developed and fertile, whereas in two-rowed barley only the

central flower of the three is fully developed and fertile.

Observe as a consequence of the mode of development of six-rowed barley that the side corns on each spikelet have a twisted form. This unsymmetrical shape is readily observed if the lateral grains are detached from the spikelet and examined on the ventral side. If two-rowed barley and the central corns of six-rowed barley are examined in a similar manner it will be found that the corns are developed symmetrically. After making these observations it is easy for a student to determine whether a sample of threshed barley belongs to the two-rowed or six-rowed class.

The student has now studied the more conspicuous characteristics of the barley corn, the development of the barley corn from the barley flower, and the leading features which distinguish the two-rowed from the six-rowed barleys. It is now desirable that he should study the distinguishing characteristics of the four leading types or sub-species of barley which are met with in ordinary technical experience. He should commence by making himself familiar with the different varieties of cultivated barley mentioned in the following table. Six sub-species are described in the table, but the two sub-species, *Hordeum intermedium* and *H. decipiens*, are not met with in commerce and do not demand special study :—

Genus.—*Hordeum sativum.*

Cultivated Barleys.

Hordeum distichum. Barleys of the "Chevalier" Type.	**Two-rowed Barleys.** — Middle flower on spikelet fertile; the two lateral flowers infertile. Ear *narrow*. A comparatively *long* distance between the corns. Hairs on basal bristle short in some varieties, long in others.	Well-known varieties are: Chevalier, Kinver, Hallett, Archer, Hanna, White and Black Ouchac.
Hordeum zeocriton. Barleys of the "Goldthorpe" Type.	**Two-rowed Barleys.** — Middle flower on spikelet fertile; the two lateral flowers infertile. Ear *wide*. A comparatively *short* distance between the corns. Hairs on basal bristle always long.	Well-known varieties are: Peacock or Fan Barley and Goldthorpe. (Standwell and Invincible are hybrids, but possess the external characteristics of Goldthorpe.)
Hordeum vulgare.	**Six-rowed Barleys.**—All three flowers on spikelet are fertile. Ear *narrow*. A comparatively *long* distance between the corns.	Most "Light Foreign" Six-rowed Barley is a mixture of white and blue *H. vulgare*; the "bere" or "bigg" of Scotland is also a variety of *H. vulgare*.
Hordeum hexastichum.	**Six-rowed Barleys.**—All three flowers on spikelet are fertile. Ear *wide*. A comparatively *short* distance between the corns.	Chilian Six-rowed Barley consists largely of white and blue *H. hexastichum*.
Hordeum intermedium.	**Six-rowed Barleys.**—All three flowers on spikelet are fertile, but the lateral flowers form *small corns* only.	Not ordinarily met with in commerce.
Hordeum decipiens.	**Two-rowed Barleys.**—Only *one* flower on spikelet.	Abyssinian species — not met with in commerce.

*** The classification adopted in this table is suggested by E. S. Beaven in an important paper entitled "Varieties of Barley," to which the student should refer. See *Journal of the Institute of Brewing*, 1902, viii., 547.

Compare Heads of Ripe Barley of Chevalier and Goldthorpe Types.—A marked difference will be perceived. The head of the Chevalier barley is much narrower than that of the Goldthorpe. This is due to the corns on the head of the Chevalier

FIG. 8.—(*a*) *Hordeum distichum*, or Chevalier Type of Barley; (*b*) *Hordeum zeocriton*, or Goldthorpe Type of Barley.

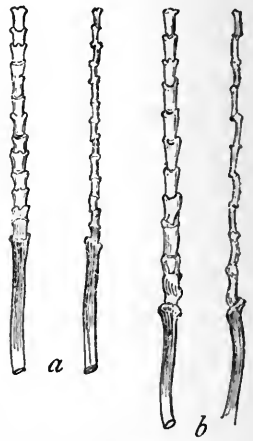


FIG. 9.—(*a*) Rachis of Goldthorpe Barley; (*b*) Rachis of Chevalier Barley.

barley forming a more acute angle with the stem or rachis than is the case with the Goldthorpe. Another distinction is that the corns on the head of the Chevalier are situated on the rachis at a greater distance apart than those of the Goldthorpe. This is rendered more apparent by strip-

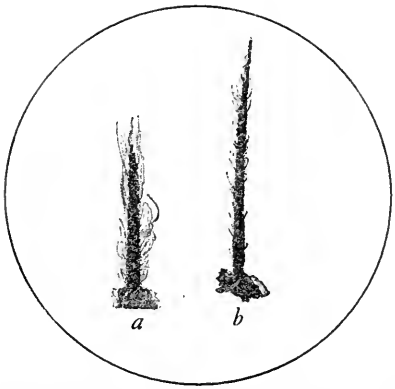


FIG. 10.—(*a*) Basal Bristle of Goldthorpe Barley; (*b*) Basal Bristle of Chevalier Barley.

ping the heads and examining the bare rachis. The internodes, or joints, from which the corns spring are thus exposed, and it will be noticed that there are from six to seven internodes in one inch of the rachis of the Chevalier, and in the Goldthorpe from nine to ten.[1]

Remove the Basal Bristle from a Corn of Goldthorpe Barley and Examine it with a Pocket Lens or a low Microscopic Power.—It will be found to be covered with long hairs, a characteristic which all barleys of the Goldthorpe type possess. On the other hand, the basal bristle of a barley of the Chevalier type (*H. distichum*) may be covered with either very short hairs or long ones. This depends on the variety of the type. The true Chevalier variety possesses very short hairs; on the other hand the Archer variety and some others possess long hairs somewhat resembling those of the Goldthorpe type (*H. zeocriton*).

Compare a Sample of Threshed Goldthorpe Barley with a Sample of Chevalier Barley.—The skin of the Goldthorpe is somewhat "greasy" in appearance, and does not adhere so closely to the corn as in the Chevalier.

Examine the Lower Ends of Goldthorpe Corns on the Dorsal Side.—A small dimple or transverse furrow may be noticed in the skin near the ex-

[1] Samples of threshed barley frequently contain portions of rachis, and these fragments will often assist the observer in determining the nature of the samples.

tremity of the lower end of some of the corns. This dimple is characteristic of the Goldthorpe type of barley, but is not always observed owing to fracture of the corn during the threshing process at the point where the dimple is situated.

FIG. 11.—*Hordeum vulgare.*

FIG. 12.—*Hordeum hexastichum.*

Compare a Head of H. vulgare with H. hexastichum. — Both are six-rowed barleys, but the head of *H. vulgare* is narrower than that of *H. hexastichum.* It will be noticed that the internodes from which the corns spring are more widely separated in *H. vulgare* than in *H. hexastichum.* Note that the general appearance of the head of *H. hexastichum* is somewhat square

in appearance, hence the name "square-headed barley" sometimes applied to it. (See Figs. 11 and 12.)

Remove the side rows of corns from heads of *H. vulgare* and *H. hexastichum*, leaving the middle rows undisturbed, thus converting the six-rowed heads into two-rowed heads.

Compare the two artificially produced two-rowed heads with heads of Chevalier and Goldthorpe barley.

It will be noticed that the general features of the head of *H. vulgare* are markedly similar to those of the Chevalier, and that the head of *H. hexastichum* bears a strong resemblance to that of the Goldthorpe. It is believed that these resemblances may indicate a near kinship in species of Chevalier and *H. vulgare*, and of Goldthorpe and *H. hexastichum*. (See Munro and Beaven, *Journal of the Royal Agricultural Society*, 1900, p. 185.)

PART II.

ANATOMY OF THE BARLEY CORN.

Bisect a soaked barley corn longitudinally through the ventral furrow with a knife. Observe that the greater bulk of the grain is composed of a white, starchy mass, called the *endosperm*, and that at the base of the corn there is a small, yellowish bud. The bud is the *embryo*, or living germ of the corn, which eventually grows into the young barley plant. Carefully remove the thick outer skin from another well-soaked corn, and note that below the thick false skin there is an extremely

thin skin, which completely envelops both the embryo and the endosperm of the grain. This is the true skin of the corn, and consists of several layers, these being the remains of the walls of the ovary and ovule, which contained the embryo-sac from which the corn has developed. (See Figs. 4 and 6.)

Note also that at the base, or germ end, of the corn, the two lodicules previously observed in the barley flower at the base of the ovary are now found compressed between the thick paleæ and the thin true skin of the corn, thus further demonstrating the relation of the barley corn to the barley flower.

Remove the Thin Skins enveloping the Embryo and Endosperm of a Barley Corn, and Observe that the Embryo may be Easily Detached from the Endosperm without Injury.—This indicates that there is no direct connection between the embryo and endosperm, but that they merely adhere together. This observation is of much importance in connection with the germination changes of the barley corn which will be studied later on.

HISTOLOGY OF THE BARLEY CORN.

Cut thin Longitudinal Sections of a Barley Corn which has been Soaked in Water for about twenty-four hours, in the Plane of the Ventral Furrow of the Corn.—This may be done with a sharp razor,

the grain being held in a piece of cork or in a hand microtome; it is, however, difficult to obtain good sections without a considerable amount of practice. Sections for permanent mounting are best obtained by employing a freezing microtome,

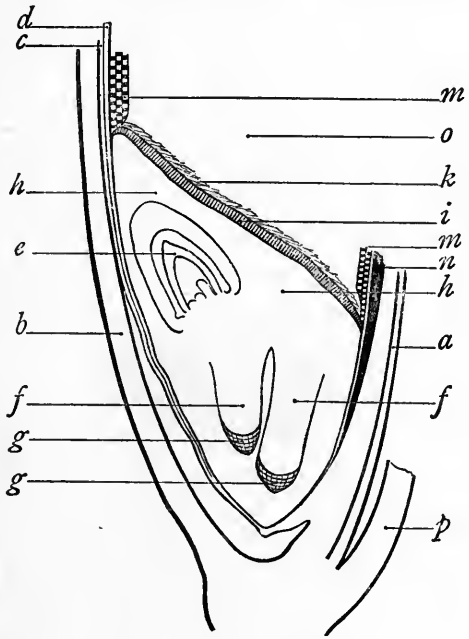


FIG. 13.—Diagram of a Longitudinal Section through the Germ End of a Barley Corn. (*a*) Palea Superior; (*b*) Palea Inferior; (*c*) Pericarp; (*d*) Testa; (*e*) Plumule; (*f*) Radicle; (*g*) Root-cap; (*h*) Scutellum; (*i*) Absorptive Epithelium of Scutellum; (*k*) Emptied and Compressed Cells of Endosperm; (*m*) Aleurone Cells of Endosperm; (*n*) Funicle; (*o*) Starch-containing Cells of Endosperm; (*p*) Basal Bristle.

the barley having been previously soaked in gum and water.

In order to render sections of barley transparent for examination with the microscope, they should

be dehydrated by means of absolute alcohol, and cleared by transferring to clove oil. They can then be examined in the latter medium, or mounted permanently in xylol and Canada balsam.

Examine the prepared sections under the microscope, first with a low-power objective (1 or 2 inch), and afterwards with a higher power of $\frac{1}{6}$ or $\frac{1}{8}$ inch.[1]

[*N.B.*—It is very desirable when a student is commencing the microscopic examination of an object which is new to him, that he should first employ a low-power objective in order to familiarise himself with the general features of the object before proceeding to study its more minute features with high power lenses.]

First note that the thick outer covering of the paleæ of the grain and the two thin true skins, called the pericarp and testa, enclose both the embryo and endosperm. Then study the embryo of the barley corn, which is the future young plant lying dormant in the seed, using Fig. 13 as a guide. Observe the appearance and position of the *plumules*, which eventually develop into the leaves of the young plant. Note the position and appearance of the *radicles* or undeveloped roots, and also the *root-caps* situated at the ends of the radicles. Identify and study the part of the embryo called

[1] See H. Brown and Morris, "Researches on the Germination of some of the Gramineæ" (Structure of a Grain of Barley), *Journ. Chem. Soc.*, 1890, lvii., p. 461.

the *scutellum*, and specially note that part which is known as the *absorptive epithelial layer*. It is the layer of elongated cells which bounds the scutellum on the side which is pressed against the starchy endosperm of the barley corn. The cells of this layer, sometimes called the *palisade cells*, have most important functions and play a leading part in the feeding of the young embryo when it commences to develop into a young plant.

Study the Endosperm.—It will be noticed that it is composed of two distinct types of cells. Nearest the skin will be observed a triple layer of thick-walled, square-shaped cells. These are called the *aleurone* cells. The rest of the endosperm is composed of much larger cells of irregular shape bounded by very thin walls. These are the starch-containing cells, and under a high power and proper illumination the starch granules will be observed lying closely packed within the cell walls. Later on, when the student examines stained and specially prepared transverse sections of barley, he will learn more regarding both the starch-containing cells and the aleurone cells.

Attention should also be given to a layer of compressed and empty cells which lie between the scutellum and the endosperm, but which form an integral portion of the latter. During the early development of the corn they contained starch

granules, but during its later development the starch was absorbed by the embryo.

Cut Transverse Sections of a Barley Corn in a Similar Manner to the Longitudinal Sections already made.—These sections should be cut as thin as possible, and it is desirable to prepare a dozen or so and preserve them in chloroform water for future use.

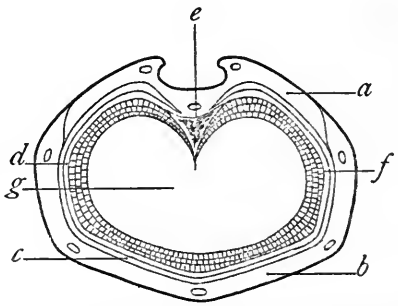


FIG. 14.—Diagram of a Transverse Section through a Barley Corn. (*a*) Palea Superior; (*b*) Palea Inferior; (*c*) Pericarp; (*d*) Testa; (*e*) Funicle; (*f*) Aleurone Cells; (*g*) Starch-containing Cells.

Dehydrate a Section in Alcohol and Examine it under the Microscope in Clove Oil or when mounted in Balsam.—The thick outer coverings, or paleæ, enclosing the grain will now be seen in cross section. The points at which the palea on the dorsal side of the grain overlaps the palea on the ventral side will be recognised. The five little ridges which run along the dorsal side of a barley corn and give it a slightly angular appearance will also be noticed as being due to five vascular bundles,

FIG. 15.—Photomicrograph of Transverse Section through a Barley Corn, showing the Funicle.

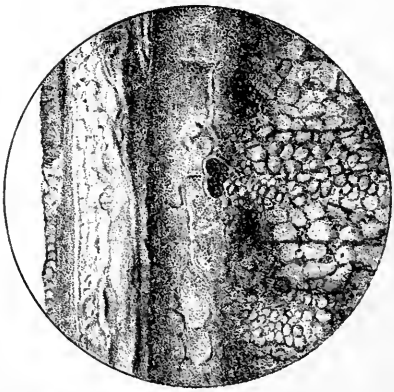


FIG. 16.—Photomicrograph of Section of Skins and Endosperm of Barley Corn (highly magnified).

or veins, in the palea inferior, which is now seen in cross-section.

The two true skins, the pericarp and testa, are also very clearly shown in the transverse section. Note how they continue round the grain until they arrive at the ventral furrow where they appear to be merged together and lost in a conspicuous dark brown spot called the funicle. This spot represents the point where the ovule was originally attached to the ovary of the barley flower before it developed into a corn (see Fig. 4, p. 5).

Within the thin skins of the transverse section, the triple layer of aleurone cells already noticed in the longitudinal section will be again observed. Within these lie the thin-walled starch-containing cells, forming the greater bulk of the endosperm. In general arrangement these cells will now be seen to radiate from near the centre of the grain.

Cut a Transverse Section of one of the Green Corns which are usually abundant in Samples of Six-rowed Barley.—Dehydrate the section, mount it in clove oil or balsam, and examine it under the microscope. Observe that the contents of the aleurone cells are coloured blue. The colour of the aleurone cells seen through the yellow outer skin of the corn is the cause of the green appearance of the corn originally observed. A green colour is a natural characteristic of certain varieties of two-rowed and six-rowed barleys and is not an indication of unripeness.

The student, after working through the studies described above, which should have given him a

good general knowledge of the structure of the barley corn, must now proceed to study some special points in the anatomy of the corn.

Note the Position in which Starch Granules occur in the Endosperm of Barley.—Transfer a thin transverse section of barley to a very dilute solution of iodine in a watch-glass, and allow it to remain in the liquid until the section is stained a distinct blue colour. Mount the section in water on a glass slide, cover with a thin glass, and examine first with a low and afterwards with a high power. The position of the blue-stained starch granules is now distinctly seen. It will be noticed that the aleurone cells contain no starch granules, and that these occur only within the thin-walled cells which compose the larger part of the endosperm. In these cells the starch granules lie very closely packed together.

Show that the Starch-containing Cells contain Protoplasmic Matter enveloping the Starch Granules.—In order to demonstrate this clearly, the starch granules must be removed without disturbing the protoplasm. This can be done by means of the action of *saliva*, as this secretion contains an enzyme—ptyalin—which is able to dissolve starch, but has no action on protoplasmic matter. Prepare 3 or 4 c.c. of saliva by washing out the mouth with a very little water. (As the secretion appears to be more active before meals it is better to obtain it then.) Filter the solution into a test-tube, place

several thin transverse sections of barley in the liquid, and keep it at a temperature of 46° C. for three or four hours by immersing it in a vessel of water maintained at the desired temperature. (If the solution is kept for more than a few hours, which may be necessary, a little chloroform water should be added to prevent the growth of micro-organisms.) When the action of the ptyalin is complete the original white appearance of the section, due to the presence of starch granules, will have disappeared. Now, place one of the starch-freed sections in a very dilute solution of *eosine* to stain the protoplasm, transfer the section to a slide, and examine it under the microscope. It will be noticed that the thin-walled cells are filled with a network of red-stained protoplasmic matter which originally enclosed the starch granules. The cavities in which the starch granules formerly lay will be clearly seen. A permanent mounting of the section in balsam should be made after first *very gradually* dehydrating the section with alcohol of increasing concentration.

PART III.

EXPERIMENTS CONNECTED WITH THE TECHNICAL STUDY OF BARLEY AND OTHER CEREALS.

Study the Microscopic Appearance of Some of the Commoner Kinds of Starch.—The starches of barley, wheat, potato, rice and maize are suitable for this study.

Transfer a little of the starch to a drop of water on a glass slide, cover with a thin glass and examine with a high power. Observe the characteristic appearance of the different kinds of starch granules and make a drawing to scale of each. If the starch used has been obtained direct from the grain it is desirable, after examining in water alone, to stain with a little iodine solution; as the starch granules alone are stained blue, this differentiates them from other organic matter which may be present. By this means also the mixture of very small granules of starch with much larger ones, as in the case of barley starch, will become more evident.

Prepare a Sample of Starch from Barley or Other Grain.—Grind about 100 grms. of the grain to a very fine powder and mix with cold water. Separate the starch from the husk and tissue of the grain as far as possible by filtering the milky liquid through very fine muslin. Allow the starch to subside from the filtrate, pour off the liquid, and

purify further by repeated washing with cold water and decantation. Digest the starch with 0·5 per cent. caustic potash solution for twenty-four hours, wash well, and drain the starch on a filter. Transfer to an unglazed porcelain plate and air-dry at the temperature of the room.

Determination of the Moisture in Barley and Other Cereals.—This determination must be conducted on a ground sample of the corn. First clean the mill by grinding through it some of the corn to be tested. Reject this sample. Then grind from 3 to 5 grms. of the corn and transfer the whole to a stoppered drying-tube the weight of which has been previously ascertained. Weigh the tube and its contents, transfer to a water-oven, remove the stopper and dry at 100° for four or five hours. Replace the stopper, transfer to a desiccator until cool and weigh. Return the tube to the water-oven for an hour or two, cool and weigh again, as before. These operations must be repeated until the weight remains constant within one or two milligrams. Now calculate from the weights obtained the percentage of moisture lost.

A more rapid estimation of the moisture may be obtained by drying in a hot-air oven kept at a constant temperature of 102° C., but care must be taken that this temperature is not exceeded.

The student should determine the moisture in kiln-dried as well as undried samples of barley, in order to demonstrate that an apparently perfectly

dry barley contains from 10 to 12 per cent. of moisture. It is important that he should recognise that this amount of moisture is natural to a barley in perfect "condition," and that it is the presence of moisture in excess of this amount which produces the different degrees of "want of condition" in barley.

Determination of the "Vitality" or Germinative Power of Barley.—The student should experiment with Coldewe's, or other form of germinator, on various samples of barley. The germinative power of barley is often regarded from two points of view —its germinative "energy" and its germinative "capacity". Germinative energy is expressed by the percentage number of corns which vegetate in a definite time, generally taken as three days. Germinative capacity is expressed by the percentage number found capable of germinating, irrespective of time.

Determination of the "Weight" of Barley.—In this country the term "weight" as applied technically to barley is understood to refer to the weight in pounds of a bushel measure of the grain. The student can gain some experience in the laboratory of the bushel weights of different kinds of barley by weighing them in the miniature bushel of the instrument known as the "chondrometer". But the results obtained must be regarded more as comparative values than reliable determinations of the actual bushel weight of the corn.

Another meaning of the expression "weight" as applied to barley refers to the weight of a certain number (usually 1,000) of corns. This method of comparing the weight of different barleys, is employed more frequently on the Continent than in this country, and has the merit of giving the true average weight of the corns, which is not expressed accurately by the bushel weight. The latter is much influenced by the shape of the corns, and, consequently, by the different manner in which they arrange themselves in the measure.

Count out 200 average corns from several samples of different barleys, including English Chevalier and Smyrna, or other "light foreign" barley, and weigh them. Express the results as weights in grams of 1,000 corns. Compare the weights of the English and Smyrna barleys, and note that the relation they bear to each other differs very considerably from the relation of their bushel weights found by the chondrometer.

Determination of the Specific Gravity of Barley.—In a well-matured, mealy barley its white friable endosperm is permeated with minute spaces containing air, which diminish its specific gravity. In a badly matured, steely barley the hard endosperm contains fewer air-spaces, and consequently its specific gravity is greater than that of the mealy endosperm. A comparison of the specific gravities of the endosperms of different barleys is therefore a measure of their relative tenderness. But in technical work it is not practicable to obtain a sufficient number of skinless endosperms in order to determine their specific gravity, and recourse must therefore be had to a determination

of the specific gravity of the whole barley corns. This may be effected by observing their displacement-volume in a liquid like toluene which does not readily penetrate the interior of the corn.

Weigh out accurately about 50 grms. of the barley, and after noting the exact weight, transfer the corns to a dry 100 c.c. flask. From an accurately graduated burette run toluene slowly into the flask, shaking it to disentangle any air which may be confined in the mass of barley corns. When the toluene reaches the 100 c.c. mark, note the volume of toluene which has been run into the flask. If the temperature of the toluene in the flask and in the burette is kept constant, the difference between the volume of toluene run into the flask and 100 c.c. represents the displacement volume of the barley. Divide the weight of this volume, considered as water, into the weight of the barley taken, and the specific gravity of the barley will be obtained. But it should be noticed that the specific gravity of the barley obtained in this manner is influenced by the air confined within the skins of the barley and that the proportion in volume of this air is liable to vary in different types of barley. Hence the above method of experiment is not reliable if used for comparing different types of barley, and can only be used with advantage for the purpose of comparing the relative mealiness of samples of barley of the same type.

As toluene is a volatile, easily inflammable liquid, experiments with it must not be conducted near a flame.

Technical Examination and Valuation of Barley for Malting Purposes.—This is a convenient time for the student to gain some experience in the valuation of barley for malting purposes. Personal instruction by an expert is absolutely necessary.

PART IV.

THE CHANGES IN BARLEY DURING GERMINATION.

Steep about 100 grms. of English barley in water for forty-eight hours, and change the steep water twice each day.

Keep the first highly coloured steep water in a beaker, and note how rapidly it commences to decompose on standing. Water extracts from barley matter which readily putrefies, and hence arises the necessity to change the steep water in the malt-house frequently.

After the barley has been steeped for about two days, examine the corns. If they are moistened throughout, but not saturated with water, the barley is sufficiently well steeped.

Determination of Moisture in the Steeped Barley.—Weigh about 10 grms. of the steeped barley in a drying bottle. Place in a water-oven and dry very slowly with the door of the oven open until most of the moisture is evaporated. Finally dry at 100° until the weight is constant. Calculate the percentage of water lost. This will give some idea of the amount of water retained by barley when steeped in the malt-house.

Study of the Changes in Barley during Germination.—Spread the rest of the steeped barley (see above) in a thin layer in some suitable form of germinator to start it into growth. Proper

attention must be given in order to keep it under conditions of temperature and moisture which favour regular and slow growth.

Observe from day to day the appearance of the growing corn and make a series of sketches showing the gradual development of the rootlets and the acrospire. Note also how the starchy part of the endosperm is modified in character as the growth of the embryo proceeds, and observe that this modification commences in the part of the endosperm which is nearest to the embryo, and gradually spreads towards the far end of the grain.

At intervals during the germination of the barley make longitudinal sections of a corn for microscopical examination. A freezing microtome should be employed, if possible, for making the sections. Prepare the sections for examination in the manner already described for preparing sections of barley (p. 14).[1]

Note that the first sign of the action of the growing embryo on the endosperm is the solution of the compressed layer of emptied cells adjacent to the scutellum (Fig. 13). This action is produced by the enzyme cytase which is secreted by the scutellum of the growing embryo. Cytase transforms the insoluble cellulose of the cell-walls into a soluble sugar.

[1] See H. Brown and Morris, "Researches on the Germination of some of the Gramineæ" (The Visible Changes which occur in the Embryo and Endosperm during Germination), *Journ. Chem. Soc.*, 1890, lvii., p. 466.

As the development of the embryo proceeds, note the disappearance of the cell-walls of the starch-containing cells of the endosperm which are nearest to the embryo, and the consequent liberation of the starch granules. Observe how the action spreads through the endosperm as the growth of the embryo proceeds. Note that it is this action which transforms the hard barley corn into the friable grain of malt.

Remove with the point of a needle a trace of the starch nearest to the scutellum of a partially grown barley corn, and examine it in water with a high-power lens. Note that many of the starch granules are "pitted"—*i.e.*, show signs of being partially dissolved. This is due to the action of the enzyme diastase, secreted by the scutellum of the growing embryo. Diastase converts the insoluble starch granules into soluble sugar, and it is mainly by its action that the growing embryo obtains its necessary carbo-hydrate food from the endosperm.

Remove carefully the thick and thin skins from a well-grown barley corn, and note that the layer of aleurone cells can be readily detached from the rest of the endosperm. In a non-germinated barley corn this is not the case. The readiness with which the aleurone cells separate in a germinated corn is due to the fact that the aleurone cells, as well as the scutellum of the embryo,[1] secrete cytase, and the action of this enzyme dissolves

[1] See H. Brown and Escombe, "On the Depletion of the Endosperm of *Hordeum vulgare* during Germination," *Proc. of Royal Soc.*, vol. lxiii., 1898.

the walls of the starch-containing cells adjacent to the aleurone layer, and so ruptures the connections between it and the starch-containing cells of the endosperm. Hence the aleurone cells, as well as the cells of the epithelial layer of the scutellum, contribute towards the modification of the endosperm.

Demonstrate that the Embryo will Grow to a Limited Extent when Separated from its Endosperm and Supplied with Water only, and that it will Grow Freely under the Same Circumstances if it is Supplied with Suitable Food.[1]—Soak some barley corns (preferably six-rowed Chilian or Smyrna)[2] in water for twenty-four hours, and remove the embryos from about a dozen of the corns without injuring them. This may be easily done by turning back the skin of the corn at the germ end and lifting the exposed embryo with the point of a blunt knife.

Sterilise two small Petri dishes, each containing a flat piece of porous unglazed porcelain, about 2 square inches in area. Also prepare some sterilised water, and a sterilised 3 per cent. solution of cane-sugar. Pour sufficient sterilised water into one of the dishes to cover the bottom and thoroughly moisten the unglazed porcelain. Repeat this operation with the other dish, using the cane-sugar solution. Now place four or five of the excised

[1] See H. Brown and Morris, "Researches on the Germination of some of the Gramineæ" (Culture of Embryos of Barley on Water), *Journ. Chem. Soc.*, 1890, lvii., p. 482.

[2] The embryos of these barleys are not so tender as those of two-rowed barleys.

barley embryos on the flat surface of each of the pieces of porcelain with the scutella of the embryos resting on the moistened surface of the porcelain. The embryos are then in a position to obtain the moisture they require in order to start them into growth. Place the covers on the dishes, and keep them in a moderately cool place. In about twenty-four hours signs of growth in the embryos will be noticed in both experiments. Protrusion of the roots is first observed, and shortly afterwards the plumules commence to elongate. During the first two or three days the growth of the embryos proceeds in both experiments at about the same rate, showing that water alone, as well as cane-sugar solution, stimulates germination. But by-and-bye the growth of the water-fed embryos ceases owing to lack of food, whilst the sugar-fed embryos continue to increase to a considerable size, and the young plants commence to develop chlorophyll.

If the cane-sugar solution contains suitable mineral nutriment and a little nitrate of potash, perfect plants can sometimes be reared.

When conducting the above experiments note that every precaution must be adopted to prevent the growth of moulds and bacteria, which readily attack the exposed embryos and check their development.

Demonstrate that the Embryo of the Barley Corn secretes Diastase during its Development.—Prepare 100 c.c. of a 1 per cent. solution of *soluble*

starch in water. Add to the solution when cold 7 grms. of gelatin, and heat gently in a water-bath until the gelatin is dissolved. Sterilise the solution in a steam steriliser, and when it is still hot pour it into a sterilised Petri dish, which is sufficiently deep to hold easily $\frac{1}{3}$ to $\frac{1}{2}$ an inch of the liquid. Place the cover on the dish and allow the gelatin solution to cool until it commences to show signs of setting. Now take several excised barley embryos which have been previously prepared, and place them on the surface of the setting jelly so that their scutella are in perfect contact with it.

FIG. 17.—Experiment Demonstrating the Secretion of Diastase by the Growing Embryo of a Barley Corn.

Put the covered dish in a cool place, and in three or four days the embryos will have developed considerably. Remove the cover from the dish, and with a knife make two long parallel cuts in the jelly, one on each side of an embryo almost touching it, and place the thin slice of jelly obtained in this manner on a white porcelain plate, and brush over it a solution of iodine in iodide of potassium. The iodine will stain most of the gelatin a deep blue colour, owing to the soluble starch present, but in the portion of the jelly situated immediately below the scutellum of the embryo a cup-shaped colourless part will be noticed. This is due to the

soluble starch originally contained in this portion of the jelly having been converted into sugar by the action of the diastase secreted by the scutellum of the embryo.

If the jelly below another embryo is examined in a similar manner, but at a later date, it will be noticed that the action of the diastase gradually extends as the embryo develops.

The growth of liquefying bacteria must be guarded against as far as possible in this experiment, or the success of the experiment may be interfered with.

Demonstrate that Carbon Dioxide is Generated during the Growth of the Barley Corn.—Arrange four flasks as in the accompanying illustration so

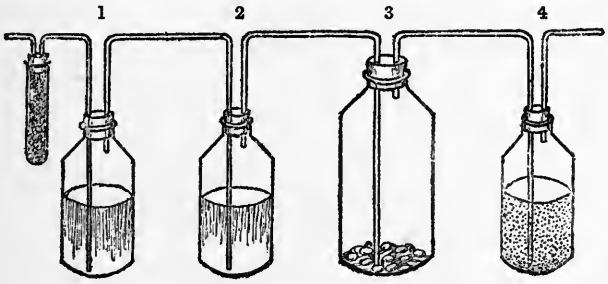


FIG. 18.—Apparatus for Demonstrating that Carbon Dioxide is Generated during the Respiration of Germinating Barley.

that the first contains a solution of caustic soda, the second a solution of barium hydrate, the third some germinating barley corns, and the fourth a solution of barium hydrate. Connect an aspirator to the fourth flask, and draw a current of air slowly through the flasks. As the air enters the first flask

it is deprived of the carbon dioxide it contains, and this is shown by the solution of barium hydrate in the second flask remaining clear. But as the current of air passes through the flask containing the germinating barley, it mixes with the carbon dioxide generated by the respiration of the growing corn and conveys it to the fourth flask, where its presence is indicated by the formation of a copious white precipitate of barium carbonate.

Demonstrate that Oxygen is Absorbed during the Growth of the Barley Corn.—Introduce a quantity of actively germinating barley into a large, wide-mouthed stoppered bottle, and place in the bottle a small dish containing a strong solution of caustic soda in order to absorb the carbon dioxide generated by the respiration of the growing corn. Close the bottle with the stopper and allow it to remain overnight in a warm room. Remove the stopper and introduce a lighted taper into the bottle. Observe that the taper is extinguished, owing to the oxygen originally present in the bottle having been removed by the respiration of the germinating barley.

Preparation of Malt Diastase.—Digest 100 grms. of air-dried or pale-dried malt with 250 c.c. of 20 per cent. alcohol for four hours and then filter. Add strong alcohol to the filtrate so long as a flocculent precipitate of diastase is formed. Allow the precipitate to subside and pour off the supernatant liquid. Wash the precipitated diastase

by decantation with a little strong alcohol, and afterwards transfer the precipitate to a hardened filter. Wash the precipitate on the filter repeatedly with small quantities of absolute alcohol and transfer both the filter and precipitate to a vacuum desiccator. By dehydrating the diastase in this manner it may be obtained as a light granular powder.

In order to test the activity of the prepared diastase dissolve about 0·1 grm. of it by rubbing it in a small mortar with about 5 c.c. of water, and transfer the mixture to 50 c.c. of a 1 per cent. solution of soluble starch. Keep the mixture at a temperature of 60° (140° F.). The activity of the diastase will be shown by the gradual disappearance of the iodine starch reaction, due to the transformation of the starch into sugar.

When preparing diastase it is desirable to carry on the process as rapidly as possible, since prolonged contact of the diastase with alcohol tends to destroy its activity. The first precipitate of diastase may be redissolved in water and reprecipitated by alcohol if a purer preparation is desired.

Action of the Enzyme Cytase.—Prepare an extract of air-dried malt, or of oats, by digesting 25 grms. when finely ground with about 70 c.c. of cold water for three or four hours. Filter the extract, which contains the enzyme cytase. Immerse in the extract several very thin transverse sections of a barley corn, and place the vessel containing the solution in an incubator at a temperature of 25° to 30°. In four or five hours examine one of the sections with a microscope, and it will be

observed that the walls of the starch-containing cells are swelling and gradually dissolving away. A complete disintegration of the cell walls should be obtained in about twelve hours, but if the experiment is continued for this length of time it is advisable to add a little chloroform water to the cytase extract in order to prevent the growth of micro-organisms.

If malt is employed for the purpose of preparing the extract used in this experiment, it is essential that it should not have been kiln-dried, for cytase is destroyed by the heat of the kiln. For a similar reason kiln-dried oats should not be used.

An Experiment to Determine the Loss in Weight occurring during the Conversion of Barley into Malt.—The loss in weight which occurs in the malting of any special steeping of barley is often difficult to determine in the ordinary routine of malt-house work, as it entails the weighing-up of the finished malt under conditions which are usually inconvenient. A measure of the loss may, however, be made in the laboratory in the following manner :—

Average samples of the barley, as steeped, and of the screened malt made from the barley, are required.

Count out 1,000 corns from each sample, and weigh them accurately. The figures thus obtained express the weight of the barley and the weight of the malt obtained from a similar weight of barley. Calculate from these figures the yield of malt from 448 lbs. (or 1 qr.) of the barley. Calculate also from the same figures the percentage loss in weight of the barley when malted.

Note that the loss determined is only in part due to a loss of solid matter during malting, because barley always contains considerably more moisture than finished malt. In order to estimate the true loss of solid matter due to root-growth, respiration, and extraction by steeping water during the malting process, determine the moisture in the samples of barley and malt. From the figures thus obtained and those previously determined, the amount of solid matter lost is readily calculated.

TECHNICAL EXAMINATION OF MALT.

This is a convenient time to study the ordinary technical methods of examining and valuing malt. Practical experience under the guidance of an expert is essential. As a general guide to the student his attention is directed to the following points :—

1. The general appearance of the sample, and the kind and character of the barley from which the malt has been made.

2. The "modification" or relative tenderness of the sample.

3. The "condition" or relative dryness of the sample.

4. The flavour of the sample.

5. The regularity of growth. (In order to practise the eye in judging this character it is useful to separate 200 corns from a sample of malt into four classes of "still corns," half-grown, three-quarters

grown and "grown out," and express the results as percentages.)

6. The broken corns present.

7. The mouldy corns present.

The "Sinker" Test.—This test is extensively used in the technical examination of malts, and gives valuable information when employed intelligently.

Count out 200 corns and place them in a glass full of water at a temperature of about 15° (60° F.). After moistening the corns by gentle agitation, remove at once all the corns which float. Note the position of the corns which have sunk; some will probably rest upright, indicating that the corns are steely ended; others may lie flat, which usually indicates dead or very steely corns. Remove the "sinkers," count them and examine each corn separately after dividing it longitudinally with a knife. Class the corns as (1) dead; (2) steely; (3) steely-tipped; (4) vitreous; (5) corns which have sunk through damage to skin, etc.

In cases of importance it is desirable to take 500 or 1,000 corns for this test, as 200 is too small a number on which to base a satisfactory judgment. It is very important that the malt should be in good condition when subjected to the "sinker" test, as the specific gravity of malt is raised very considerably when it becomes "slack".

Determination of the Specific Gravity of Malt.—The specific gravity of malt may be determined by the toluene method, as already described for barley (see p. 25). Some idea may be gained of the relative tenderness of malts of similar nature by this means, but the results are not very reliable.

PART V.

THE CHEMICAL ANALYSIS OF MALT.

Determination of the Moisture in Malt.—This determination is carried out in a similar manner to the determination of moisture in barley, described on p. 23. As malt takes up moisture from the air very rapidly it should be exposed as little as possible, particularly after grinding. Subject to the complete drying of the malt, the drying process should be carried on for as short a time as possible, as after prolonged heating malt is inclined to gain slightly in weight.

Determination of the Ash of Malt.—Place about 5 grms. of the ground malt in a weighed platinum dish, and re-weigh in order to determine the quantity of malt taken. Heat the dish gently with a Bunsen flame until the malt is completely charred. Transfer the dish to a muffle furnace, and heat until the ash is colourless. Weigh and calculate the percentage of ash obtained. The ash should be examined qualitatively for potassium, phosphoric acid and silica, of which it principally consists.

Determination of the Extract of Malt.—Before this very important determination is attempted the student must make himself familiar with the ordinary method of determining the specific gravity of liquids by means of the specific gravity bottle and the balance.

Determination of the Specific Gravity of Liquids. —A 50 c.c. specific gravity bottle with a perforated stopper and a counterpoise are required.

Clean the bottle thoroughly, and after washing with distilled water, rinse it out with a little strong alcohol. Warm the bottle gently over a flame and suck air through it by means of a glass tube until it is quite dry. Allow it to cool in a desiccator. Ascertain by means of a balance which turns distinctly with ·0005 grm., if the bottle together with its stopper is of the same weight as the counterpoise. If not, the counterpoise must be accurately adjusted by adding to, or subtracting from, its weight.

The capacity of the bottle must now be determined by filling it with distilled water at the standard temperature of 15·5° (60° F.), and weighing. The following is a convenient and rapid way of carrying out this operation :—

Take about 70 to 80 c.c. of distilled water in a 100 c.c. flask, introduce a thermometer with a wide degree-scale into the water, and bring the water exactly to the temperature of 15·5° (60° F). Pour the water rapidly into the specific gravity bottle, which must be held by the neck during this and the following operations. Close the bottle with its stopper, so as not to include any air bubbles, and at once wipe the top of the stopper, which must not afterwards be touched. Dry the bottle with a soft cloth as quickly as possible, and place it at

once on the pan of the balance and weigh quickly. In order to obtain manipulative skill in this process, the student should repeat the operations of filling the bottle with water, and weighing until he can obtain consecutive weights varying by not more than ·001 grm.

If it is found that the bottle contains exactly 50 grms. of water, the specific gravity of any other liquid may now be obtained by multiplying by 20 the weight of the liquid it will contain, for the capacity of the bottle is equal to that of 50 grms. of water at 15·5°, and 50 × 20 = 1,000, the usual standard to which the specific gravity of liquids is referred. It rarely happens, however, that a specific gravity bottle contains exactly 50 grms. of water. Although it is possible to make a bottle correct by grinding the stopper, this operation is very tedious, and it is preferable, if the error of a bottle does not exceed ·02 grm., to use the error as a constant correction and, as the case may be, either add it to or subtract it from every weighing of the bottle.[1] The specific gravities of the solutions likely to be met with by the student do not differ sufficiently from that of water to introduce

[1] The error should be redetermined at frequent intervals. Also the student should be careful to employ the same thermometer in all his determinations, as ordinary laboratory thermometers are often very inaccurately graduated. If the same instrument is always used, even considerable inaccuracy in graduation will not introduce any appreciable error.

an appreciable error when adopting this convenient plan.

If the error exceeds ·02 grm. the bottle should be exchanged for a more accurate one, or, if preferred, the weight of the water contained by the bottle may be used as a divisor for the weight of the contents of the bottle when filled with any other liquid whose specific gravity is required. This method gives accurate results with a bottle of any capacity, but the calculation involved becomes troublesome when many specific gravity determinations have to be made.

The method of determining specific gravities recommended above is sufficiently accurate for ordinary purposes of technical analysis, but for special work it is desirable to use a Sprengel tube, or a pyknometer with a thermometer stopper and side tube.

Preparation of the Malt Mash (*Heron's Method*). —Weigh out roughly about 55 grms. of malt and grind it through a mill to a moderately fine meal. Transfer the whole of the ground malt to a balance, and weigh 50 grms. accurately. (This method of weighing ground malt should always be adopted in order to obtain an average sample. The weighing must be done rapidly, as ground malt is very hygroscopic.)

Transfer the 50 grms. of malt to a 500 c.c. copper beaker, and mash it with 350 c.c. of water at a temperature of 69° (156° F.). Cover the beaker

with a clock-glass and place it in a water-bath regulated by a thermostat, so that the mash is kept at a constant temperature of 66° (151° F.). After one hour transfer the whole of the mash to a 515 c.c. graduated flask by means of a wide-necked copper funnel, taking care that the water used for washing-in the "grains" does not raise the volume above the 515 c.c. mark. Cool the contents of the flask to 15·5° (60° F.), and make up the volume to the 515 c.c. mark by the addition of water. Mix the solution thoroughly by agitation, and filter about 250 c.c. of the wort through a dry filter into a dry vessel.

Determine the specific gravity of the filtered wort by the method already described, and calculate the pounds of extract derived from 1 qr. (336 lb.) of the malt.

The calculation is made in the following manner: The total volume of the mash is made up to 515 c.c. on the assumption that the volume displacement of the residue of "grains" is 15 c.c., and therefore that the total volume of the wort is 500 c.c. As 50 grms. of malt were mashed, the extract from this weight of malt is contained in 500 c.c. of the wort. Hence the extract from 10 grms. of malt is present in 100 c.c. of the wort. Assuming that a specific gravity of 1027·5 has been found for a wort, it is evident that 100 c.c. of this wort weighs 102·75 grms. Now, this volume of wort contains the extract from 10 grms. of malt, and

therefore the solution weight of the extract, *i.e.*, the "wet" extract from 10 grms. of malt, amounts to 102·75 – 100, or 2·75 grms. It is obvious that the extract from 10 lb. of malt must be 2·75 lb., therefore the proportion 10 : 2·75 :: 336 : 92·4 indicates that 92·4 lb. extract has been obtained from 336 lb. or 1 qr. of the malt.

Extract Determined on the Boiled Wort.—Measure accurately 100 c.c. of the filtered wort already prepared, in a 100 c.c. flask, and transfer it to a beaker. Rinse the flask with a little water and add the rinsings to the beaker. Boil and note the "breaking" of the wort due to the coagulation of the precipitated albuminous matter. After the volume of the liquid is reduced by boiling to about 70 c.c., pour it back into the 100 c.c. measure, washing the beaker carefully and transferring the washings to the flask. Take care that the washings do not raise the volume above the 100 c.c. mark. Cool the liquid to 15·5° (60° F.), make up to 100 c.c. and filter through a dry filter. Take the specific gravity of the wort and calculate the extract as before. Compare the extract found with that of the unboiled extract and note the small difference caused by the removal of the coagulable albuminous matter.

Calculation of the Dry Extract from the observed "Wet" Extract.—One hundred c.c. of a solution containing 1 grm. of dry malt extract weighs 100·40 grms., indicating that 1 grm. of malt extract

weighs 0·4 grm. when in solution. If, therefore, the weight of 100 c.c. of a wort minus 100 grms. is divided by 0·4, the weight of dry malt extract present in 100 c.c. of the wort is obtained.[1] For instance, the specific gravity of the unboiled malt extract already referred to was 1027·5, and therefore 100 c.c. weighs 102·75 grms. If the weight of the extract when in solution, *viz.*, 2·75 is divided by 0·4, the quotient 6·875 is the weight of dry extract present in 100 c.c. of the wort. As the dry extract was derived from 10 grms. of malt it follows that the malt yielded 68·75 per cent. of dry extract when mashed.

Calculation of weight of Dry "Grains".—From the calculated dry extract it is possible to calculate the amount of dry "grains" left after mashing the malt. 100 grms. of malt have been shown to yield 68·75 grms. of soluble dry extract, therefore 100 grms. – 68·75 grms. = 31·25 grms. not accounted for. This weight represents the insoluble "grains," and the moisture in the malt previous to mashing. If the moisture amounted to 3 per cent., the approximate amount of dry grains left after mashing 100 grms. of malt would be 31·25 – 3 = 28·25 per cent. of dry grains. The weight of "grains" produced during the mashing of 1 quarter (336 lb.) of malt can then be calculated if desired.

[1] The divisor for wort solids varies slightly with the specific gravity and nature of the wort; but a constant factor of 0·4 is sufficiently accurate for the purposes of the experiment.

It should be observed, however, that the above method of calculating the weight of dry "grains" yields a result which is a little too low, because a small fraction of the weight of the dry extract used in the calculation is not derived from the malt, but is due to fixation of water during the hydrolysis of the starch of the malt.

Influence of Fine and Coarse Grinding on Extract.—In order to study this effect two samples of the same malt, one ground to a coarse meal, and the other as finely as possible, should be mashed under similar conditions, and the extracts of both determined. The finely ground sample will be found to yield the higher extract, and the difference will be greater with a hard "steely" malt than with a tender sample. This experiment indicates the desirability of establishing standard conditions of grinding for the purpose of systematic malt examination.[1]

Criticism of the Method of Determining Extracts described above.—The method just described is the one most commonly employed in this country for determining extracts, but although it is a very useful method it is open to the objection that the means by which the measure of the volume of the wort is obtained is not very accurate. It has already been mentioned that it is based on the assumption that the insoluble "grains" from 50 grms. of malt occupy a volume of 15 c.c., and, therefore, if the total volume of the mash is made

[1] A thoroughly satisfactory malt mill has yet to be introduced.

up to 515 c.c. the volume of the wort is 500 c.c. But the displacement volume of the dry grains from all malts is not the same, because the proportion of the insoluble husk and cellular tissue to the soluble matter in malts varies. The difference is not great, and has little or no influence on the result when the method is used for comparing the relative yield of extract from malts of a similar type, but when the extracts of malts of widely differing types, such as Smyrna and English, are compared, the results may be affected appreciably. No doubt also a more accurate volume than 15 c.c. might be selected with advantage to represent the average displacement of the "grains" from 50 grms. of malt, but at present it is advisable to follow the plan most commonly adopted, as uniformity of method in the determination of malt extracts for ordinary technical purposes is very desirable.

Determination of Extract by Weighing the Mash.—The method of mashing employed is similar to that used in the previous process, but the volume of the wort obtained is calculated from the weight of the water used in mashing and the specific gravity of the wort obtained.

Weigh the empty beaker in which the mash is to be made, and then mash in it 50 grms. of the ground malt with about 350 c.c. of water at the same temperature and for the same time as before. Cool the mash and ascertain the total weight of

the beaker and the mash. Subtract the weight of the beaker and malt from the total weight, and the difference represents the weight of the water used in mashing. Filter the mash and determine its specific gravity in the usual manner.

The method of calculating the extract obtained will be seen in the following example :—

Weight of empty beaker	= 102·0 grms.
,, of malt (containing 4 % moisture)	= 50·0
	152·0
Total weight of beaker and mash	= 532·0 grms.
,, ,, ,, ,, and malt	= 152·0
Water used in mashing	= 380·0
Moisture in 50 grms. malt	= 2·0
Total water in mash	= 382·0

Sp. gr. of wort found = 1035·5. Therefore 100 c.c. weighs 103·55 grms. As 0·4 is the weight of 1 grm. of malt extract in solution, $\frac{3{\cdot}55}{{\cdot}4}$ = 8·875 grms. dry extract in 100 c.c. Therefore 103·55 − 8·875 = 94·675 grms. water in each 100 c.c. wort.

Then if 94·675 grms. water = 100 c.c. of wort,

$$\frac{382{\cdot}0 \text{ (total water in mash)} \times 100}{94{\cdot}675} = 403{\cdot}5 \text{ c.c. wort in mash.}$$

Now 100 c.c. wort contains 3·55 grms. wet extract; therefore

$$\frac{403{\cdot}5 \times 3{\cdot}55}{100} = 14{\cdot}324 \text{ grms. wet extract from 50 grms. of malt.}$$

Therefore $\frac{14{\cdot}324 \times 336}{50}$ = 96·2 lb. extract obtained from the malt.

The accuracy of this method of determining the extract of a malt turns mainly[1] on the degree of accuracy with which the factor 0·4 represents the solution weight of 1 grm. of dry malt extract.

This factor is not quite correct in all cases, as the true factor varies slightly for malt worts of different gravities and of varying composition, but the error introduced by its use is very small, and may be ignored in technical analysis.

A method of determining the percentage of dry extract yielded by a malt which is very generally employed on the Continent is based on the process just described.

Two methods of determining extracts of malts other than those already described are sometimes used. One consists in mashing the malt in the usual way, filtering and washing out the extract to a known volume. A description of this process will be found in L. Briant's *Laboratory Text-Book for Brewers*, second edition, p. 123. The second process, advocated by Dr. A. L. Stern, is described in the *Journal of the Federated Institutes of Brewing*, vol. i., p. 448.

Determination of the "Full Theoretical" Extract.—Those methods already described are employed to determine the extracts of malts when mashed under conditions approximating to those in a brewery; but the whole of the possible extract is not obtained by such means, because even in the case of tender malts a small amount of the starch present is not hydrolysed, whilst with hard malts a large amount often escapes conversion.

A method frequently employed to obtain the total available, or "full theoretical" extract consists in treating the ground

[1] Probably a small error is introduced when attempting to correct for the moisture in the malt.

malt previous to mashing with a cold water infusion of oats. Ripe oats (not kiln-dried) contain a considerable amount of the enzyme cytase, and a cold water infusion containing this enzyme is employed to act on the malt for the purpose of liberating the starch contained in the unmodified portions of the malt by dissolving the cell walls enclosing the starch granules.

Digest 50 grms. of oats, rather finely ground, with 250 c.c. of cold water, for three or four hours, and filter. Mix 100 c.c. of the filtrate with 50 grms. of the ground malt experimented with, and allow the mixture to stand at the room temperature for from eighteen to twenty-four hours. Mash with 250 c.c. hot water, so as to obtain an initial heat of 66° (151° F.), and keep at this temperature for one hour. Transfer the mash to a 515 c.c. flask, and after cooling, make up to the mark and filter, and proceed to determine the specific gravity of the wort in the usual manner. Before calculating the extract a correction must, however, be made for the specific gravity of the oat extract used in the experiment. Determine the specific gravity of the oat extract used, and subtract one-fifth of its excess gravity above 1,000 from the specific gravity of the malt extract. The extract derived from the malt can then be calculated in the usual manner.

If the ordinary laboratory extract of the same malt has been determined, the difference between the two extracts is a measure of the modification of the malt. The so-called "co-efficient of modification" may be obtained by calculating the ordinary extract as a percentage on the "full theoretical" extract. For instance, if the ordinary extract of a malt is 87·6, and the "full theoretical" extract 95·7,

$$\text{then } \frac{87{\cdot}6 \times 100}{95{\cdot}7} = 91{\cdot}5,$$

the "co-efficient of modification" of the malt, and the value is comparable with the "co-efficient of modification" of any other malt found in a similar manner.

Determination of the Extract of "Flaked" and Prepared Grain.—A determination of the extract

of "flaked" or other forms of grain, prepared for use in the mash tun without previous conversion, may be made in the following manner :—

Mash 16·66 grms. of the prepared grain with 33·33 grms. of pale malt in the usual manner. Also mash 50 grms. of the pale malt alone. Determine in the usual manner the extract obtained from 336 lb. of the malt alone, and from 336 lb. of the mixture of malt and prepared grain. Now subtract two-thirds of the extract derived from the malt alone from the extract of the mixed malt and grain, and it is obvious that the remaining extract is derived from one-third of a quarter (112 lb.) of the grain.[1] Multiply the amount by 3 to obtain the extract derived from 336 lb. of the grain.

Determination of the Extracts of Black and Brown Malts.—The extracts of these kinds of malt may be obtained by the same method as the one used for prepared grain. The colour of the malt should be measured by means of the Lovibond "tintometer," in accord with some determined standard of dilution.

Determination of the Extract of Raw Grain.—Weigh out 16·66 grms. of the finely ground grain, and 33·33 grms. of ground pale malt of which the extract is known, and put the grain and the malt

[1] The extract of the malt alone must be determined very carefully, for any error accumulates on the determination of the extract of the prepared grain. This remark also applies to the calculation of brewery extracts when mixed mashing materials have been used.

into separate beakers. Add 250 c.c. of cold water to the raw grain, slowly raise the heat of the mash in a water bath to 100° (212° F.), and keep at this temperature for thirty minutes. Cool the contents of the beaker to 70° (158° F.), and add about 5 grms. of the ground malt. Stir the mixture well and allow it to stand for ten minutes in order that the gelatinised starch may be liquefied by the diastase of the malt. Raise the heat of the mash slowly over a gas flame to the boiling point, and boil for one hour with frequent stirring. Add 100 c.c. cold water to the boiled mash, reduce its temperature to 69° (156° F.), and mash in the rest of the malt. Keep the temperature at 66° (151° F.) for one hour and then proceed as before (p. 51) to determine the extract yielded by the raw grain.

It is desirable to use a beaker of Jena glass for boiling the mash in the above experiment.

When determining the extract of very hard grain it is sometimes advantageous, in place of boiling the mash, to digest it in an autoclave at a pressure of 7 to 10 lb. The operation should be conducted in a wide-mouthed stoppered bottle, with the stopper tied down in order to prevent loss of the mash during cooling in the autoclave, which is otherwise likely to occur.

Determination of the Acidity of Malt.—Digest 50 grms. of the ground malt with 300 c.c. of cold distilled water for three hours, stirring the mash occasionally. Filter, and estimate the acidity in 150 c.c. of the filtrate by means of $\frac{N}{20}$ ammonia

solution, using litmus-paper as an indicator. Calculate the acidity as lactic acid, and express the result as acid found in 100 grms. of malt.

This method is the one usually adopted in malt analysis, but it is a very unsatisfactory one, as the acid reaction of most malts is not due to free acid but chiefly to acid phosphates for which litmus is a very insufficient indicator.

KH_2PO_4 is acid to litmus until about half the salt is changed to K_2HPO_4, when the mixture is acid to blue and alkaline to red litmus; *i.e.*, its action is amphoteric. Consequently, as malt contains both acid and secondary phosphates, titration with litmus as an indicator gives no reliable measure of any form of acidity, and the value of the determination, if it possesses any, is solely empirical.

The acidity of malt due to acid phosphates (and free acid, if it contains any) may be determined in a fairly satisfactory manner with caustic soda, using phenolphthalein as indicator. (See Fernbach, "Phosphoric Acid in Barley and Malt," *Journal of the Federated Institutes of Brewing*, vol. ii., 1896, p. 128.)

Determination of the Diastatic Power of Malt (Lintner's Method).—

(1) Digest 25 grms. of the ground malt with 500 c.c. of distilled water for four hours at 18° (65° F.), stirring every half-hour. Filter about 50 c.c. of the malt extract perfectly bright.

(2) Prepare 100 c.c. of a 2 per cent. solution of soluble starch (see p. 65) in distilled water. Measure 10 c.c. of this solution into each one of a series of eight test-tubes which have been recently washed and well drained.

(3) Measure by means of a pipette exactly 0·1 c.c. malt extract into the first of the tubes. Into the second tube measure 0·2 c.c., into the third 0·3 c.c., and so on, until all eight tubes of starch solution contain malt extract in regularly increasing amounts. Note the exact time when the malt extract is added, and place the tubes in a water bath kept at 21° (70° F.), having previously shaken them in order to thoroughly mix the added malt extract with the starch solution. Allow the tubes to stand for *exactly one hour*, during which time the amount of the soluble starch transformed is directly proportional to the quantity of malt extract present. Now add to each tube 5 c.c. of Fehling's solution (see p. 78), mix by shaking, and then heat the tubes in a boiling water bath for ten minutes. Remove the tubes and allow them to stand until the precipitated cuprous oxide has settled. Examine the tubes and ascertain by means of their colour the amount of malt extract which has produced enough sugar in one hour to exactly reduce all the copper in 5 c.c. of Fehling's solution. Then, assuming that 0·1 c.c. malt extract corresponds to a diastatic power of 100, and x equals the quantity of extract determined by experiment,

$$\frac{0{\cdot}1 \times 100}{x} = \text{the diastatic power of the malt.}$$

But a correction for the reducing sugars present in the malt extract must be made. If this is not determined directly (which is rarely necessary), 1·5

should be subtracted from the diastatic power found.

When pale malts are examined, the malt extract should be added in quantities of ·10, ·15, ·2, ·25 c.c., etc., as subdivision into the larger amounts mentioned above is not sufficiently accurate for malts of high diastatic power.

When the student is learning this process he should always make his experiments in duplicate, as the manipulation necessary to obtain good results requires a considerable amount of practice. Special attention must be given to the cleansing of the test-tubes, which should be treated shortly before use with a little strong nitric acid and then thoroughly washed with water. The tubes should be drained by inverting them in a clean beaker; they must not be placed on pegs, which are always liable to be dirty. The soluble starch used must be perfectly free from acid. All the minor details in connection with this process must receive very careful attention, or reliable results will not be obtained.

Determination of the Diastatic Power of Barley.—Ungerminated barley contains a form of diastase which hydrolyses soluble starch, but, unlike malt diastase, acts very slowly on ordinary starch paste. The diastatic power of barley is determined in a similar manner to that of malt, and experiments should be made with two or three different kinds of barley. (See Baker, *Journ. Chem. Soc.*, 1902, lxxxi., p. 1177.)

Determination of the "Non-coagulable Albuminoids" in Malt.—The Kjeldahl method of esti-

mating nitrogen in organic substances is employed in this determination, and if the student is not already familiar with the process he must practise it before proceeding with the analysis of malt.

The method consists first in converting the nitrogen of the substance under examination into ammonia by treatment with strong sulphuric acid, and then in estimating the ammonia formed by distilling it into a known volume of standard acid. Standard solutions of $\frac{N}{20}$ sulphuric acid and ammonia are required. Prepare a normal solution of sulphuric acid in the usual manner. Dilute 25 c.c. of this solution to 500 c.c. with ammonia-free water, thus making a $\frac{N}{20}$ sulphuric acid. To prepare a $\frac{N}{20}$ ammonia solution add 3 c.c. strong ammonia to 500 c.c. water, titrate the solution with $\frac{N}{20}$ acid and dilute until correct. Methyl orange should be used as indicator.

Determine the Nitrogen in Asparagin.—Dissolve 0·30 grms. of finely powdered crystals of asparagin, dried by pressing between layers of filter-paper, in a little water, and make up the volume of the solution to 50 c.c. Measure 5 c.c. of the solution into a 100 c.c. Jena glass flask or beaker, and evaporate it to dryness on a water bath. Add 10 c.c. pure strong sulphuric acid and 5 grms. potassium sulphate to the dried residue, and heat the mixture very strongly until the solution is decolourised. Transfer to the distilling flask of a Kjeldahl dis-

tilling apparatus with 200 c.c. ammonia-free water. Add as rapidly as possible a strong solution of caustic soda (sp. gr. 1300°) to the solution until it is distinctly alkaline, and at once connect the distilling flask with the distilling apparatus. Proceed to distil the solution into a flask containing 25 c.c. $\frac{N}{20}$ sulphuric acid. When about half the volume of the solution has distilled over the operation is complete. The contents of the flask containing the $\frac{N}{20}$ sulphuric acid must now be titrated with $\frac{N}{20}$ ammonia solution, using methyl orange as an indicator. The difference of the volume of the $\frac{N}{20}$ ammonia solution used, from the original 25 c.c. $\frac{N}{20}$ acid taken, represents the volume of $\frac{N}{20}$ acid neutralised by the ammonia distilled over from the mixture in the flask. Before attempting to calculate the amount of nitrogen in the asparagin originally taken, it is necessary to make a correction for the ammonia which may have been present in the reagents used. An experiment with the reagents alone is made in a similar manner to the one already described, and the amount of $\frac{N}{20}$ acid neutralised by the ammonia present in the reagents is used as a correction. When fresh reagents are employed it is always necessary to re-determine the correction.

To calculate the amount of nitrogen found in the asparagin taken, the number of c.c. of $\frac{N}{20}$ acid neutralised, less the correction for materials, is

multiplied by ·0007, the nitrogen equivalent of 1 c.c. $\frac{N}{20}$ sulphuric acid. As 0·03 grm. of asparagin was taken, the percentage of nitrogen obtained is readily found. The known amount of nitrogen in crystallised asparagin is 18·67 per cent., and the result obtained by experiment should be within 0·2 per cent. If the result is not satisfactory the experiment should be repeated until accuracy is obtained.

Determination of the "Non-coagulable Albuminoids" in Malt. — Digest 25 grms. of the finely ground malt in 250 c.c. distilled water for three hours at about 15·5° (60° F.), stirring the mixture occasionally. Filter, and measure 100 c.c. of the filtrate into a suitable beaker or flask, and boil for about twenty minutes to throw out of solution all the coagulable nitrogenous bodies. Transfer the solution and precipitate to a 100 c.c. measuring flask and, after cooling, make up to the original volume of 100 c.c. Filter, and measure 10 c.c. of the filtrate into a Jena glass flask or beaker. Evaporate to dryness on the water bath, and add 10 c.c. sulphuric acid and 5 grms. potassium sulphate. Heat the mixture, at first gently, until the first violent action is over, and afterwards strongly, until it is decolourised. Transfer to a distilling apparatus, and proceed as already described in the analysis of asparagin. The nitrogen found, after making the necessary correction, is the amount of nitrogen from 1 grm. of malt; from

this the amount from 100 grms. of the malt is at once obtained. On the assumption that all the nitrogen found is combined as albuminoids, this number should then be multiplied by the factor 6·3.

The factor 6·3 is based on the consideration that the average amount of nitrogen contained in proteids is about 16 per cent.; but the use of the factor 6·3 should be considered as a conventional arrangement, for the nitrogen in proteids varies very considerably, and, moreover, much of the soluble nitrogen of malt exists in combination as amides and bodies other than proteids, concerning which we have very little exact knowledge at present. This, however, does not detract from the value of the determination of "non-coagulable albuminoids" when used comparatively in the analytical examination of malts.

When the student is learning the process described above he should conduct his experiments in duplicate in order to check the accuracy of his manipulation.

Determination of the "Ready-formed Carbohydrates" of Malt.—Digest 25 grms. of the finely-ground malt with 250 c.c. distilled water for three hours at 15° to 18° (60° to 65° F.). Filter, and measure 100 c.c. of the filtrate into a suitable beaker or flask, and boil it for about twenty minutes to throw out of solution all the coagulable bodies. Transfer the solution and precipitate to a 100 c.c. measuring flask, and after cooling make up to the original volume of 100 c.c. Filter, and determine the specific gravity of the filtrate. Calculate the amount of dry solids in 100 c.c. of the

filtrate by using the factor 0·386, which is assumed to represent the weight of 1 grm. of these solids when in solution.[1] The amount of dry solids obtained is derived from 10 grms. of malt, and therefore ten times the amount represents the total solids derived from 100 grms. of malt.

The method usually adopted to determine the amount of "ready-formed carbo-hydrates" present in the total solids is as follows: It is assumed that the total solids are a mixture of "non-coagulable albuminoids," soluble ash, acid, and "ready-formed carbo-hydrates". Then, if the albuminoids, ash, and acid have been determined experimentally, the sum of the quantities found is subtracted from the total solids, and the difference is supposed to represent the "ready-formed carbo-hydrates" of the malt.

If the albuminoids, ash, and acid are not determined experimentally, an average correction for these constituents of the total solids may be made in the following manner: If the total solids are 18·5 per cent., 3·5 is subtracted, and for each variation of 0·7 per cent. of solids from this amount 0·1 is added to, or subtracted from, 3·5 previous to its employment as a correction.

The results of determinations of "ready-formed carbo-hydrates" obtained by this method must not be regarded as accurate measures of quantity, but they are of value in malt

[1] 0·386 is the factor for cane-sugar, but a portion only of the "ready-formed carbo-hydrates" of malt is cane-sugar.

analysis for purposes of comparison. The same purposes would, however, be attained equally well and by simpler means if the "total solids" only were determined, but as the method of estimating the "ready-formed carbo-hydrates" just described is very generally adopted, it is better at present to conform to common usage for the sake of uniformity.

It should also be noticed that in the preparation of the cold water extract of malt for the above process it is assumed that diastase has no action on starch during the three hours in which the malt is soaking in cold water. This is not quite accurate, for a slight but perceptible action on the starch takes place. If the cold water mash is made feebly alkaline the action of the diastase is arrested, and a more accurate measure of the "total solids" is obtained, but it is not usual to employ alkali.

Determination of the Soluble Ash and the Colour of Malt.—Both these determinations may be made on the 10 per cent. cold water extract prepared for the determination of the "ready-formed carbo-hydrates".

1. *Determination of the Soluble Ash.*—Evaporate in a weighed platinum dish 25 c.c. of the boiled and filtered solution prepared for the "ready-formed carbo-hydrate" determination. Ignite the dry residue gently with an ordinary Bunsen flame until it is thoroughly carbonised, and afterwards heat in a muffle furnace until the residual ash is quite white. Cool in a desiccator and weigh. Calculate the amount of ash found as a percentage on the original malt.

2. *Determination of the Colour of the Malt.*—Determine the colour in the one-inch cell of a Lovibond "tintometer" by means of the series of

yellowish-brown tinted glasses known as Series No. 52. Reflected light from the porcelain reflector of the tintometer should be employed.

Some analysts prefer to determine the colour of malt in an ordinary hot mash of known specific gravity, and refer it by calculation to a standard gravity of 1055·5° (20 lb.).

Analyse the colour of the malt by means of the standard red and yellow glasses of the tintometer, and express the result in terms of red and yellow.

The "Saccharification" Test.—This test is intended to measure the time in which complete saccharification of a malt mash takes place when the mash is made under normal conditions.

Ten grms. of the ground malt are mixed with 100 c.c. of water at 68° (154° F.) and kept in a water bath at 66° (151° F.), the mash being stirred occasionally. In fifteen minutes about 5 c.c. of the mash are withdrawn and filtered through a small filter, and the filtrate, after cooling, is tested with iodine solution for the presence of starch. If starch is found the test is repeated at intervals of five minutes until the reaction of iodine with starch is no longer observed. The time taken for the complete saccharification of the mash is then noted.

When the student is familiar with the methods of malt analysis described above he should analyse several samples of malt of different character, making the following determinations :—

Extract.
Moisture.
Diastatic power.
Non-coagulable albuminoids.
Acidity.

Soluble ash.

Colour.

Ready-formed carbo-hydrates.

At the same time he should make a physical examination of the malts as described on p. 37. The results of the analyses should be tabulated by the student and submitted to his instructor for criticism.

SECTION II.

PRINCIPLES OF THE MASHING PROCESS.

PART I.

The Following Course of Experiments Constitutes a Study of Some of the Carbo-Hydrates Concerned in Wort Production, and Introduces the Student to the Special Methods Employed in their Examination.

Action of Water on Starch. — The student during his previous course of work has examined starch granules from various sources under the microscope (p. 22). He should now study the action of water of gradually increasing temperature on starch.

Mix about 4 grms. of potato starch with 100 c.c. of cold water in a Jena glass beaker, and heat the mixture slowly, keeping it constantly stirred with a thermometer. Note that the starch granules commence to swell as the temperature of the water approaches 65° (149° F.), and as the temperature rises further they gelatinise completely and mix with the water, forming a semi-transparent viscid mixture called starch paste.

Boil the starch paste and note that it still remains viscid. Cool the paste, and its viscosity is much increased.

Boil 1 or 2 c.c. of the starch paste with 5 c.c. of Fehling's solution. No red precipitate of suboxide of copper is formed, showing that starch paste does not reduce cupric oxide.

Preparation of Soluble Starch.—Introduce about 50 grms. of potato starch into a 500 c.c. flask, and half fill the flask with a 7·5 per cent. solution of hydrochloric acid made by diluting 125 c.c. of the concentrated acid to 500 c.c. with distilled water. Allow the starch to digest with the dilute acid at the ordinary room temperature for seven or eight days. The acid should then be poured off and the starch washed repeatedly with distilled water by decantation until the *granules* no longer give an acid reaction when placed on blue litmus-paper. One or two drops of dilute ammonia should then be added, and the starch again washed until every trace of ammonia is removed. Drain the starch thoroughly on a filter, and spread it on filter-paper to air-dry at a temperature of about 25° (77° F.).

Examine the starch granules under the microscope, and note that in general appearance they are very similar to granules not treated by acid. Dissolve about 3 or 4 grms. in 100 c.c. of boiling water, and note that the solution differs from an ordinary starch paste in being limpid.

Study the Action of Dilute Sulphuric Acid on

Starch.—Prepare 200 c.c. of a 3 per cent. starch paste. When preparing starch paste note that the starch must not be introduced into boiling water as a dry powder, for under these conditions it forms lumps which do not gelatinise properly. The dry starch should first be mixed with a little cold water to form a thin cream, and the mixture should then be run slowly into boiling water with constant stirring until gelatinisation is complete.

When the starch paste is boiling gently, add 2 grms. of strong sulphuric acid diluted with about 5 c.c. of water, and continue heating the mixture. The first noticeable change is the very rapid liquefaction of the starch paste owing to its conversion into "soluble starch".

At intervals of five minutes from the addition of the acid to the starch paste transfer two quantities of 5 c.c. each into two test-tubes. Cool one tube and add just enough of a dilute solution of iodine to develop its full colour. Add 10 c.c. of Fehling's solution to the other tube and heat it in a boiling water bath for ten minutes. Arrange the tubes in consecutive series, and note that, as the hydrolytic action of the sulphuric acid on the starch paste proceeds, the iodine-blue reaction of the original starch gives place to purple, reddish-purple, red, and finally to no colour; at the same time the precipitate of red oxide of copper in the series of tubes containing Fehling's solution increases rapidly in amount.

This experiment indicates that the hydrolytic action of sulphuric acid on starch paste is first to change it into a more soluble form (soluble starch), and afterwards to form a compound which gives a red reaction with iodine (erythro-dextrin), which is subsequently changed to a body which gives no colour with iodine. The increasing amount of red oxide of copper in the tubes containing Fehling's solution indicates the gradual production of a reducing substance (dextrose) as the starch is hydrolysed to its final point.

Prepare the Sugar, Dextrose, which is the Final Product of the Acid Hydrolysis of Starch.—Mix 100 grms. of potato starch with a little cold water to a thin cream and pour it slowly into 1,000 c.c. of boiling water in which 50 grms. of oxalic acid have been previously dissolved. When the starch is poured slowly into the boiling acid solution it is rapidly converted into soluble starch, and there is no difficulty in preparing a 10 per cent. solution in this manner.

Digest the solution for ten hours at 100° (212° F.) in a steam steriliser to completely hydrolyse the starch to dextrose. Neutralise the hot acid solution by adding an excess of calcium carbonate, and so remove the oxalic acid from the solution as insoluble oxalate of calcium. Filter, and concentrate the filtrate to a thick syrup on a water bath. Dissolve the syrup in twice its volume of 93 per cent. alcohol and allow the solution to cool

slowly. If a syrup falls out of solution on cooling, the alcohol is too strong, and a few drops of water should be added and the solution again heated to redissolve it. When the cooled solution no longer deposits any syrup add a crystal of dextrose and set aside to crystallise.

After crystallisation is complete, which may take six or seven days, drain the crystals of dextrose and dry by spreading them on a porous earthenware plate. To recrystallise the dextrose, dissolve the dried crystals in half their weight of water and add to the resulting syrup twice its volume of boiling 93 per cent. alcohol. Set the alcoholic solution aside to crystallise and dry the resulting crystals as before.

Study the Hydrolysis of Starch by Acid quantitatively, and show that 100 parts of Starch yield 111·1 parts of Dextrose when completely Hydrolysed, according to the Equation, $C_6H_{10}O_5 + H_2O = C_6H_{12}O_6$.—Dry 6 grms. of pure potato starch in a weighing bottle at a temperature of 80° (176° F.), gradually rising to 110° (230° F.), until the weight is constant. Mix the known weight of dry starch with a little water to a thin cream, and carefully transfer the whole of the mixture to a beaker containing 70 c.c. of boiling water in which 2·5 grms. of oxalic acid have been dissolved. Boil gently until solution of the starch is complete. Then wash the whole of the solution into a flask, taking care that the total volume of the solution closely

approximates to 100 c.c., thus ensuring that the solution contains the proper strength of acid, *viz.*, 2·5 per cent.

Digest the solution in a steam-steriliser at 100° (212° F.) for fourteen hours. When the digestion is completed, neutralise the hot solution with calcium carbonate, filter, and wash the precipitate thoroughly. Evaporate the filtrate and washings to about 70 c.c., transfer to a 100 c.c. flask, cool and make up to 100 c.c.

Determine the specific gravity of the solution, and its opticity in the 100 mm. tube of a polarimeter. Calculate from each of the above determinations separately the amount of dextrose which has been obtained from 100 parts of dry starch.

(At present the methods of quantitatively determining carbo-hydrates by means of their solution density and optical activity have not been studied, but they are described in the following pages, and the student should have had time to become familiar with them before the termination of the experiment just described.)

Determination and Use of the "Solution Weights" of Carbo-Hydrates.—In the ordinary course of experimental work with carbo-hydrates it is often necessary to ascertain the amount of substance present in a solution; but the usual chemical method of evaporating a known volume of the solution to dryness and weighing the residue can rarely be employed, owing to the obstinate

manner in which most carbo-hydrates retain water, and to their liability to decompose on long-continued heating. In order to dry them without decomposition they must be submitted to very special and lengthy processes.[1]

To avoid these difficulties, the specific gravity of a solution of a carbo-hydrate may be used to determine the weight of substance present, if the solution weight of the carbo-hydrate is known.

Experiment.—Dry some pure cane-sugar in a water-oven at 100° (212° F.). Weigh out exactly 10 grms. of the sugar, and dissolve it in about 50 c.c. of water in a small beaker. Transfer the solution to a 100 c.c. flask, and after cooling to 15·5° (60° F.), make up the volume to the mark with water.

Determine the specific gravity of the solution (which should prove to be 1038·64). The weight of the solution in excess of 1,000, *viz.*, 38·64, is the solution weight of 10 grms. of sugar, and therefore the solution weight of 1 grm. is 3·864. It is obvious, therefore, that the use of the factor 3·864 as a divisor for the specific gravity of the solution less 1,000, determines the weight of dry cane-sugar in 100 c.c. of the solution. It should be noted, however, that different carbo-hydrates possess dif-

[1] See Brown, Morris and Millar, "Examination of the Products of Starch-Hydrolysis by Diastase," *Journ. Chem. Soc.*, 1897, lxxi., p. 76.

ferent solution factors, and that the solution factors vary slightly with the concentration of the sugar solutions. The factors for different concentrations of well-known carbo-hydrates met with in ordinary technical work will be found on referring to Table I., p. 187.

As an exercise, dissolve a known weight (4 or 5 grms.) of pure dry cane-sugar in about 50 c.c. of water, and make up the volume of the solution to 100 c.c. at 15·5° (60° F.). Determine the specific gravity of the solution, and, after referring to the table for the correct solution factor for the specific gravity found, calculate the amount of cane-sugar in 100 c.c. of the solution, and compare with the known weight of cane-sugar originally taken.

Determine the Solution Density of Dextrose.—This sugar cannot be dried in the oven in the ordinary manner, as it does not part with the whole of its water of crystallisation under such conditions. Special means for drying in a vacuum must be employed.

Introduce 10 grms. of the pure sugar into the tared flask of a "Lobry de Bruyn" apparatus.[1] Dry in a vacuum, first at a temperature of 70° (158° F.) until it has lost most of its moisture, and then raise the heat slowly to 105° (221° F.). When the weight has become constant, dissolve the sugar in the flask in about 90 c.c. of water. Note the weight of the solution in the flask, and after determining its specific gravity, calculate its volume. As the volume of the solution and the weight of the dry dextrose it contains are now known, calculate the weight of dextrose in 100 c.c.

[1] See note, p. 70.

Then $\frac{\text{Sp. gr.} - 1{,}000}{\text{grms. per 100 c.c.}}$ = the solution factor of dextrose for the concentration of the solution experimented with. Compare the result with the solution factor given in Table I. for a dextrose solution of similar specific gravity.

Introduction to the Use of the Polarimeter in Carbo-Hydrate Work.—The student should have at his disposal a Laurent sodium-light polarimeter, and a Schmidt and Haensch half-shadow polarimeter.

The specific rotation of an optically active carbo-hydrate is the angle through which a ray of polarised light of definite refrangibility is rotated when it traverses a column 1,000 mm. in length of a solution of the carbo-hydrate containing 10 grms. in 100 c.c. of solution. But although a column of 1,000 mm. is adopted as the standard of length to which specific rotations are referred, such a length is very rarely used experimentally; columns of 100 and 200 mm. in length are generally employed, for which the proper corrections are readily made, since the rotation of a ray of polarised light varies directly with the length of the column through which the ray passes.

Moreover it is unnecessary to observe the rotation of a 10 per cent. solution of a carbo-hydrate in order to determine its specific rotation, for the rotation of a ray of polarised light varies directly with the concentration of the solution through which it passes. Therefore, if the rotatory power

of a column of any known length of a solution containing any known amount of substance is ascertained, the specific rotation of the substance may be calculated in the following manner :—

Example.—A solution known to contain 5·50 grms. of cane-sugar in 100 c.c. is observed in a sodium-light polarimeter in a column of 100 mm., and its rotation is found to be 3·66°.

Then $3{\cdot}66° \times 10 = 36{\cdot}6°$ rotation in 1,000 mm. Therefore $\frac{36{\cdot}6° \times 10 \text{ grms.}}{5{\cdot}5 \text{ grms.}} = 66{\cdot}6°$ = the specific rotation of cane-sugar.

The rotation of a ray of polarised light by an optically active substance varies with the refrangibility or colour of the ray ; it is therefore necessary to indicate the character of the light used when expressing the specific rotation of a substance. The standard light employed is the yellow light derived from the D lines of the sodium spectrum, and specific rotations determined by this light are denoted by the sign $[\alpha]_D$.

When the light employed is the so-called "medium yellow" of the spectrum, specific rotations are denoted by the sign $[\alpha]_j$.

The relation of $[\alpha]_D$ to $[\alpha]_j$ is in the ratio of 1 : 1·111 ; therefore to convert $[\alpha]_D$ into $[\alpha]_j$ the expression $[\alpha]_D \times 1{\cdot}111$ is employed ; to convert $[\alpha]_j$ into $[\alpha]_D$ the expression $\frac{[\alpha]_j}{1{\cdot}111}$ applies. Specific rotations should always be expressed as $[\alpha]_D$, but it is necessary to grasp the relation of $[\alpha]_D$ to $[\alpha]_j$

in order to understand the at one time frequent use of $[\alpha]_j$ in literature concerning the carbo-hydrates.[1]

The scale of the Laurent sodium-light polarimeter is graduated in degrees of arc, and therefore readings with this instrument can be used directly for the purpose of determining $[\alpha]_D$, as described above. But the Schmidt and Haensch half-shadow instrument differs from the Laurent inasmuch as the scale is subdivided into divisions of an arbitrary character. The full scale of 100 divisions with which it is furnished expresses the rotation of a solution of cane-sugar of a specific gravity of 1,100 in a column 200 mm. in length—such a solution containing 26·048 grms. of cane-sugar per 100 c.c. at 17·5° (63·5° F.). When using the instrument there is, however, no necessity to take any account of this, for the divisions of the scale of the instrument bear a fixed relation to the degrees of a sodium-light polarimeter. Each single division is equal to 0·3459 of a degree of the Laurent instrument for the ordinary carbo-hydrates, *excepting cane-sugar*, for which each division of the scale is equal to 0·3469 of a degree. When, therefore, the half-shade instrument is employed to determine the specific rotation of a carbo-hydrate, multiply by 0·3459 for all carbo-hydrates other than cane-sugar, in order to convert the observed scale

[1] See Brown, Morris and Millar, *Journ. Chem. Soc.*, 1897, lxxi., pp. 84 *et seq.*

divisions into angular degrees for sodium light; in the case of cane-sugar multiply by 0·3469.

In order to convert divisions of the half-shade instrument into degrees $[\alpha]_D$ multiply by 0·3843 for all carbo-hydrates other than cane-sugar; in the case of cane-sugar multiply by 0·3854.

Determine the Specific Rotation, or $[\alpha]_D$, of Dextrose by means of the Laurent Sodium-light Polarimeter.—Dissolve 7 or 8 grms. of dextrose in about 100 c.c. of water, and boil the solution for a few minutes. Cool the solution, determine its specific gravity, and calculate by means of the proper solution factor (see Table I.) the weight of dextrose in 100 c.c. of the solution. Now observe the rotatory power of the solution in the 200 mm. tube of the Laurent instrument, and calculate the $[\alpha]_D$ of dextrose from the observed angle of rotation, and the known amount of dextrose in the solution. Compare with the known specific rotation of dextrose, $[\alpha]_D = 52{\cdot}8°$.

Determine the Specific Rotation, or $[\alpha]_D$, of Dextrose by means of the Half-shadow Instrument.—Transfer the 200 mm. tube of dextrose solution used in the previous experiment to the half-shadow instrument, and observe the number of divisions of the scale expressing the rotation of the sugar solution.

Compare the observed reading in divisions with the reading in degrees found when using the Laurent instrument, and calculate the value of a division expressed as the fraction of a degree. Compare

the result with the known value—one division = 0·3459°.

Convert the observed number of divisions into degrees by means of the factor 0·3459, and calculate the $[\alpha]_D$ of dextrose as in the previous experiment. Compare the results of the two determinations, which should be in close agreement.

The Use of a Factor other than the True Solution Factor in Determinations of the Specific Rotations of Carbo-Hydrates. — In the previous experiments on the determination of the specific rotation of dextrose, the true solution factor has been employed in the calculations. Occasionally, however, when calculating specific rotations of the carbo-hydrates, it is desirable to employ factors which differ to some extent from the true solution factors. If specific rotation is determined in this manner the solution factor employed should be expressed after the sign $[\alpha]_D$ or $[\alpha]_j$. Thus $[\alpha]_{D3\cdot86}$ indicates that 3·86 is the solution factor which has been employed in determining the particular specific rotation to which the sign refers.

Experiment. — Calculate the $[\alpha]_{D3\cdot86}$ and the $[\alpha]_{j3\cdot86}$ of dextrose from the results of the previous experiment.

Determine by means of the Polarimeter the Amount of Sugar present in a Solution when the $[\alpha]_D$ of the Dissolved Sugar is Known.—Prepare a solution of pure cane-sugar of unknown strength (5 to 15 grms. in 100 c.c.), and determine its rota-

tory power in the 100 or 200 mm. tube of the Laurent polarimeter.

From the result obtained and the known specific rotation of cane-sugar $[\alpha_D]$ 66·6°, calculate the amount of cane-sugar present in 100 c.c. of the solution. Check the result by means of the solution density method.

"Mutarotation" of Dextrose.—When the optical activity of a freshly prepared solution of dextrose in cold water is determined, it is found that the $[\alpha]_D$ of the dissolved sugar is much higher than 52·8, the recognised $[\alpha]_D$ of dextrose; but on standing at room temperature the optical activity of the solution slowly decreases, until after ten or twelve hours the value $[\alpha]_D$ 52·8 is finally reached. The explanation of this phenomenon lies in the fact that dextrose, when first dissolved in cold water, exists temporarily as a modification which possesses about twice the rotatory power of the ordinary stable form of dextrose. This bi-rotatory or "α" form is, however, rapidly converted into the stable "β" form on raising its solution to the boiling point.

Experiment.—Dissolve 6 or 7 grms. of dextrose in 100 c.c. of cold water and divide the solution into two portions. Allow one portion to remain at the ordinary temperature. Raise the other portion to the boiling point for two or three minutes and cool it again. Observe the difference in rotatory power of the two solutions when examined in the polarimeter. Allow the solutions to stand for two or three hours and examine them again. Note that the rotatory power of the boiled solution remains constant, but that the rotatory power of the other has decreased.

This experiment demonstrates that a freshly prepared solution of dextrose must be raised to the boiling point, or its bi-rotation otherwise destroyed, before determining its optical activity.

The phenomenon of mutarotation is exhibited by other

sugars than dextrose. Among these, maltose and levulose are sugars with which the student will frequently be brought into contact in the course of his experiments. A freshly prepared solution of maltose containing the "α" modification has a lower specific rotation than that of the stable "β" modification. On the other hand, the "α" modification of levulose, like that of dextrose, has a higher specific rotation than the "β" or stable modification. It will be evident from these remarks that freshly prepared solutions of both maltose and levulose must be treated in a similar manner to those of dextrose previous to examination in the polarimeter.[1]

The Cupric Oxide Reducing Power of Dextrose, Introducing the Cupric Oxide Reducing Method of Estimating Sugars.

Preparation of Fehling's solution. Two solutions are required :—

(1) *Copper Sulphate Solution*, prepared by dissolving 69·2 grms. of the pure salt in distilled water and making up the volume of the solution to 1,000 c.c.

(2) *Alkaline Tartrate Solution*, prepared by dissolving 346 grms. of Rochelle salt (sodium potassium tartrate) and 130 grms. of anhydrous sodium hydrate in distilled water and making up the volume of the solution to 1,000 c.c.

The solutions must be stored in separate bottles, and when required for use must be mixed in equal volumes.

[1] For recent views concerning the mutarotation of dextrose, see H. E. Armstrong, "Studies on Enzyme Action," *Journ. Chem. Soc.*, 1903, lxxxiii., p. 1310.

Determination of the Reducing Power of Dextrose.[1]—Weigh out about 5 grms. of dextrose, dissolve it in 100 c.c. of water, and determine the concentration of the solution from its specific gravity. (See Table I. for divisor.)

Prepare the Fehling's solution required by mixing 25 c.c. of the copper sulphate solution with 25 c.c. of the alkaline tartrate solution in a No. 5 spout beaker, and dilute the mixed solution with such a quantity of water that, with the dextrose solution, to be added subsequently, the total volume is 100 c.c. In other words, the Fehling's solution used is diluted with its own volume of liquid.

Cover the beaker containing the diluted Fehling's solution with a clock-glass, and heat in a boiling water-bath. After a few minutes, add to the Fehling's solution an accurately measured or weighed volume of the dextrose solution, and continue the heating of the solution for exactly twelve minutes. Filter off the red precipitate of cuprous oxide which is formed through a Soxhlet tube connected with a water pump, and wash it with at least 200 c.c. of boiling water, and afterwards with about 10 c.c. of strong alcohol. Place the Soxhlet tube in a water or hot-air oven and dry at 100° (212° F.). When dry, reduce the cuprous oxide in the tube to metallic copper by gently heating the tube with

[1] The standard method proposed by H. Brown, Morris and Millar is adopted. (See *Journ. Chem. Soc.*, 1897, lxxi., p. 278.)

a gas flame in a current of dry hydrogen. Allow the tube to cool in a desiccator and weigh.

Refer to Table III. to ascertain the amount of dextrose corresponding to the weight of copper found, and compare the weight found with the known weight of dextrose used in the experiment. Calculate the weight of dextrose found as a percentage on the weight of dextrose taken.

When judging the proper amount of dextrose to be taken for the reduction experiment just described, some points must be borne in mind :—

(1) The amount of copper weighed should be not less than 0·15 grm., and not more than 0·35 grm. As dextrose reduces approximately twice its weight of copper, the weight of dextrose taken should be from 0·07 to 0·17 grm. Two c.c. of a 5 per cent. solution would therefore be about the right amount to take.

(2) When it is necessary to use less than 2 c.c. of a sugar solution, the experimental error of measurement from a pipette has an appreciable influence on the accuracy of the result. In such case it is advisable to weigh the desired amount of solution accurately, and calculate the volume of the solution used from its weight and specific gravity.

(3) As Fehling's solution almost always gives a slight precipitate on heating due to spontaneous reduction, it is necessary to make a blank determination upon every fresh quantity of Fehling's solution prepared, and correct for this in all experi-

ments in which the solution is used. The amount usually varies from 1 to 3 milligrams of copper.

Preparation and Properties of Phenyl-Glucosazone.—Dissolve 1 grm. of dextrose in 50 c.c. of water, and add to the solution 2 grms. of phenyl-hydrazine dissolved in 2 grms. of 50 per cent. acetic acid. Heat the mixture in a boiling water bath, and observe that the glucosazone slowly separates from the hot solution as a dense yellow precipitate. The action is complete in one hour.

Examine the precipitate under the microscope with a $\frac{1}{4}$-inch lens, and note that it is composed of needle-shaped crystals, some of which may occur in fan-shaped aggregates. Make a drawing of the crystals. Filter off the precipitated osazone, wash with hot water, and dry at 100° (212° F.).

Note that glucosazone is very insoluble in boiling water; this characteristic assists in its identification.

The reaction of phenyl-hydrazine with the hexoses is as follows: If one molecule of phenyl-hydrazine is allowed to act on one molecule of a hexose, a normal hydrazone is formed:—

Dextrose. Phenyl-hydrazine. Hydrazone.

$$CH_2.OH(CH.OH)_4.CHO + C_6H_5NHNH_2 = CH_2.OH(CH.OH)_4.CH + H_2O$$
$$\qquad\qquad\qquad

Cane-Sugar or Saccharose. — An experiment has been made (p. 73) on the optical activity of cane-sugar.

Solution Density of Cane-Sugar.—The solution factors for cane-sugar in solutions of varying concentration are given in Table I.

Cane-Sugar does not possess Cupric Oxide Reducing Power.—Dissolve about 1 grm. of pure cane-sugar in a little water, and mix the solution with Fehling's solution under the standard conditions already described (p. 79). Heat the solution for twelve minutes. No reduction takes place.

Cane-Sugar does not Combine with Phenyl-Hydrazine to Form an Osazone.—Dissolve 1 grm. of cane-sugar in 50 c.c. of water and treat the solution with phenyl-hydrazine as described for dextrose (p. 81). Note that no osazone is formed until after prolonged heating, when a little glucosazone may be formed owing to slight inversion of the cane-sugar by the acetic acid in the solution.

Inversion of Cane-Sugar. — Cane-sugar is a disaccharide, and when hydrolysed (inverted) by the action of dilute acids or that of the enzyme invertase, is resolved into a mixture of equal parts of dextrose and levulose (invert-sugar), according to the equation :—

$$\underset{\text{Cane-sugar.}}{C_{12}H_{22}O_{11}} + OH_2 = \underset{\text{Dextrose.}}{C_6H_{12}O_6} + \underset{\text{Levulose.}}{C_6H_{12}O_6}.$$

The change may be demonstrated quantitatively

by both the optical and the reducing properties of the invert-sugar formed.

Inversion of Cane-Sugar with Acid.—Dissolve an accurately weighed quantity of about 10 grms. of dry cane-sugar in 50 c.c. of water, and transfer the solution without loss to a 100 c.c. flask, taking care that the volume of the solution and wash water does not exceed 90 c.c. Add 5 c.c. of strong hydrochloric acid, and make up the volume of the solution nearly to the 100 c.c. mark. Heat the flask in a boiling water-bath for thirty minutes, when inversion of the cane-sugar should be complete. Cool the solution, and make up the volume to 100 c.c.

(1) Determine the rotatory power of the solution at a temperature of 20° (68° F.), and calculate the amount of invert-sugar present in 100 c.c. of the solution from the known specific rotation of invert-sugar, $[\alpha]_D$ – 19·6° (at 20°).

(2) Determine the cupric oxide reducing power of the solution, using about 1·5 c.c. for reduction, and calculate the amount of invert-sugar present in 100 c.c. of the solution from the weight of reduced copper obtained.

Acid inversion of cane-sugar does not give such satisfactory quantitative results as inversion with yeast (described below), as the levulose formed is slowly acted upon and decomposed by the acid employed.

Inversion of Cane-Sugar with Yeast.—The inverting power of yeast is due to the enzyme inver-

tase contained in the yeast cells. Two methods of experiment may be adopted when yeast is employed as an inverting agent :—

(1) Prepare a solution of a known weight of cane-sugar in a 100 c.c. flask as in the previous experiment, but in place of hydrochloric acid, add 1 grm. of washed pressed yeast, and digest the solution in a water-bath at 50° (122° F.) for six hours. A temperature of 50° is employed because the fermentative power of yeast is arrested at this temperature, but its power of inversion is still retained. When the digestion is completed, raise the solution to the boiling point and cool to 15·5° (60° F.). Add a little alumina cream to facilitate clarification, make up to 100 c.c., and filter. Determine the rotatory power of the solution as in the previous experiment, and calculate the percentage yield of invert-sugar from cane-sugar which has been obtained. Compare with the calculated amount according to the equation for the inversion of cane-sugar (p. 82).

(2) Measure 50 c.c. of a solution of cane-sugar of known concentration into a flask doubly graduated to 50 and 55 c.c. and add 0·5 grm. of pressed yeast and three or four drops of chloroform. Cork the flask tightly, place it in an incubator at a temperature of about 30° (86° F.), and allow it to remain overnight. Chloroform is added because it arrests the fermentative power of yeast but does not influence its power of inversion. Remove

the cork, place the flask in a boiling water-bath and allow it to stand there until free from the smell of chloroform. Removal of the chloroform is necessary, as it reduces Fehling's solution and would affect the estimation of the invert-sugar by the reduction method if it was allowed to remain in the solution. Cool the solution, add a little alumina cream to facilitate clarification, and make up the volume to the 55 c.c. mark with water. Filter, and determine the reducing power and rotatory power of the solution, as before. When making the requisite calculations, note that a correction for the dilution of the solution from 50 c.c. to 55 c.c. is necessary.

Determine the Amount of Cane-Sugar present in Malt by Inversion with Yeast.—Weigh out 50 grms. of finely ground malt, introduce it into a flask, and add 200 c.c. of water. Make the mixture very faintly alkaline with caustic soda, and allow it to stand over night. The solution is made slightly alkaline in order to prevent the invertase which may be present in the malt from acting on the cane-sugar during the process of extraction. Filter 100 c.c. of the solution and render it very faintly acid with dilute acetic acid. This is done in order to restore the solution to a condition in which invertase is active. Observe the reading of the solution in the 100 mm. tube of the polarimeter. Now place 50 c.c. of the solution in a 50-55 c.c. flask, add 0·5 grm. pressed yeast and a few drops of chloro-

form, and proceed according to the second yeast-inversion method described above. Make a second reading of the solution in the 100 mm. tube, and after correcting for the dilution of the solution (50 c.c. to 55 c.c.), calculate from the readings made before and after inversion the amount of cane-sugar in 100 grms. of the malt.

Levulose ($C_6H_{12}O_6$).—Pure levulose may be obtained from Schering's levulose (originally prepared from invert-sugar by means of the insoluble calcium compound of levulose), by re-crystallisation from absolute alcohol. Pure levulose may also be obtained by the hydrolysis of inulin with dilute acid. (For method of preparation see abstract of paper by A. Wohl in *Journ. Chem. Soc.*, 1890, vol. lviii., p. 1087.)

Specific Rotation of Levulose.—$[\alpha]_D$ – 92·0° at 20° (68° F.). Dissolve about 5 grms. of pure levulose in 80 to 100 c.c. water, heat to boiling (see p. 78), cool and determine the concentration of the solution by means of its specific gravity. (See Table I. for solution factors for levulose.) Determine the rotatory power of the solution in the 200 mm. tube at the different temperatures of 15·5° (60° F.), 20° (68° F.) and 30° (86° F.), and calculate the $[\alpha]_D$ of levulose for the three temperatures. These observations will demonstrate that the optical activity of levulose is very sensitive to alterations of temperature, and that it is necessary when determining the rotatory power of solutions of levulose and invert-sugar to take special care with regard to the temperature of the solutions at the time of observation.

Determination of the Cupric Oxide Reducing Power of Levulose.—Determine the reducing power of about 2 c.c. of the solution of levulose according to standard conditions, and compare the result with the reduction figures for pure levulose given in Table III.

Preparation of the Osazone of Levulose.—Dilute about 20 c.c. of the levulose solution to 50 c.c. with water, and heat in

the usual way with 2 grms. of phenyl-hydrazine and acetic acid (p. 81). An osazone separates during heating with the characteristics of glucosazone. Under the microscope the crystals appear similar to those of glucosazone, and in fact the osazone of levulose is identical with glucosazone. Study the constitution of levulose and dextrose with regard to the formation of the same osazone from both.

PART II.

The Following Course of Experiments Constitutes a Study of the Hydrolysis of Starch by Diastase, and of the Products of Hydrolysis.

Preparation of Cold-water Malt Extract.—A cold-water malt extract supplies a solution of active diastase for the experiments described below. Mix one part by weight of finely ground pale dried malt with two and a half parts of cold water, allow the mixture to stand four or five hours, and filter. When cold-water malt extract is referred to in the following studies it is intended to be prepared by this method unless otherwise stated.

Study the Hydrolysing Action of Diastase on Starch.—Prepare 200 c.c. of a 3 per cent. starch paste (see p. 66). Cool to 60° (140° F.), and add 10 c.c. of cold-water malt extract, the temperature of the starch paste being kept constant. Observe the rapid liquefaction of the starch paste due to its conversion into soluble starch.

At intervals of five minutes from the addition of the malt extract to the starch paste, remove two quantities of 5 c.c. each of the solution to two test-tubes, and immerse them at once in boiling water for a few minutes to stop further action of the diastase. Proceed to treat the solutions in the tubes with iodine and with Fehling's solution, in the manner described for the acid conversion of starch on p. 66. Changes similar to those observed during the acid hydrolysis of starch will be noticed. But although the earlier chemical changes of starch hydrolysis by acid and by diastase are very similar, the final reducing sugar produced by the action of acid is dextrose, and that produced by the action of diastase is maltose.

Show that the Hydrolysing Action of Diastase is Destroyed at 100° (212° F.).—Boil 10 c.c. of malt extract for a few minutes, cool the solution, and add it to 50 c.c. of starch paste at a temperature of 60° (140° F.). Observe that neither liquefaction nor hydrolysis of the starch paste takes place.

Preparation of Maltose ($C_{12}H_{22}O_{11}$), the Reducing Sugar formed by the Action of Diastase on Starch.—Weigh out 200 grms. of potato starch, and divide it into three approximately equal portions. Gelatinise one portion in about 1,200 c.c. of boiling water in the usual manner. (If a beaker is employed for this experiment it should be of Jena glass.) Cool the starch paste to 60° (140° F.), and liquefy it by the addition of 1 c.c. of malt extract.

Raise the liquefied starch solution to the boiling point by immersing the vessel containing it in boiling water, and gelatinise the second portion of starch, as before.

Cool again to 60°, and liquefy a second time by the addition of 1 c.c. of malt extract. Raise the solution again to the boiling point and gelatinise the final portion of starch. A solution containing about 15 per cent. of soluble starch is thus procured.

Cool the solution to 55° (131° F.), add 40 c.c. of malt extract, and allow the mixture to stand over night in order to obtain the maximum conversion of the starch into maltose and dextrin. Boil, filter, and evaporate the filtrate in a porcelain dish on a water-bath until a fairly thick skin forms on the surface of the syrupy solution. Pour the syrup while hot into a flask containing 500 c.c. of boiling 93 to 95 per cent. alcohol. Part of the syrup—maltose—dissolves, and part—dextrin mixed with maltose—is precipitated. Connect the flask containing the mixture with an inverted condenser, and keep the alcohol boiling gently in a water-bath for five or six hours in order to dissolve out as much of the maltose from the dextrin as possible. Repeated agitation at this stage of the process aids the solution of the maltose.

Allow the mixture to cool and then decant the alcoholic solution of maltose from the gummy residue of dextrin into another flask. Reserve the

flask containing the residue for future experiment (see p. 93).

Proceed to connect the flask containing the alcoholic solution of maltose with a distilling apparatus, and remove the alcohol as completely as possible from the solution by heating the flask in a water-bath. Pour the thin syrupy residue into a beaker, and when cold add a little crystallised maltose. In twenty-four hours the syrup should set to a semi-solid crystalline mass of impure maltose which should then be spread on a plate of porous earthenware to dry. In order to purify the crude product by recrystallisation, ascertain its weight, and for every 100 grms. found, add 25 c.c. of water and heat until a syrup is obtained. Dissolve the syrup in 250 c.c. of hot 88 per cent. alcohol (sp. gr. 0·828) and note that at this point it may be necessary to boil the alcoholic solution with animal charcoal in order to remove colouring matter. Add a little crystallised maltose to the filtered alcoholic solution and allow it to stand. Drain the maltose which crystallises out from the alcoholic solution, wash on a filter with 95 per cent. alcohol, and dry on a porous earthenware plate.

The amount of maltose obtained may be increased by again extracting the original dextrinous residue with boiling 85 per cent. alcohol, and proceeding as described above.

Specific Rotation of Maltose $[\alpha]_D$ 138·0°.—Dis-

solve about 10 grms. of pure maltose in 100 c.c. of water, and boil the solution for a few minutes. (See p. 78 on the mutarotation of maltose.) Determine the strength of the solution by means of its specific gravity. (See Table I., for solution factors for maltose.) Determine the rotatory power of the solution and calculate from the observed angle the $[\alpha]_D$ of maltose. Compare with the known $[\alpha]_D$ of maltose.

Determination of the Cupric Oxide reducing Power of Maltose.—Determine the reducing power of a known volume of the solution of maltose by the standard method previously employed (p. 79). Maltose reduces about its own weight of copper, so that a suitable quantity of the solution for the experiment is readily determined. Compare the result of the reduction experiment with the results for maltose given in Table IV.

Preparation and Properties of Phenyl-Maltosazone.—Dissolve 2 grms. of maltose in 50 c.c. of water, and add to the solution 2 grms. of phenylhydrazine dissolved in 2 grms. of 50 per cent. acetic acid. Heat the mixture for one hour in a boiling water-bath.

Observe that the solution, when hot, remains clear in a manner very different from a solution of glucose under similar conditions (see p. 81), but on allowing the solution to cool, a yellow precipitate of an osazone is formed. Examine the precipitate under the microscope and note that the crystals

of the osazone are in the form of long flat plates. Compare with the crystalline form of glucosazone, (p. 81).

Filter off the maltosazone precipitate, wash with cold water, and dry.

Hydrolysis of Maltose by Acid.—Maltose is a disaccharide which yields dextrose on hydrolysis. (Compare with the acid hydrolysis of the disaccharide cane sugar which yields a mixture of dextrose and levulose, p. 83.)

Dissolve about 10 grms. of pure maltose in 150 c.c. of water, and determine the concentration of the solution by the specific gravity method. Measure exactly 100 c.c. of the solution into a suitable flask and add 2·5 grms. of oxalic acid. Heat the solution for fourteen hours in a steam steriliser. When digestion is complete, neutralise the hot solution with calcium carbonate, filter, and wash the precipitate thoroughly. Evaporate the filtrate and washings to about 70 c.c.; transfer to a 100 c.c. flask, cool and make up to 100 c.c. Determine the amount of dextrose from its rotatory power. Note that 100 parts of maltose yield 105·26 grms. of dextrose on hydrolysis, according to the equation :—

$$\underset{\text{Maltose.}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Dextrose.}}{2C_6H_{12}O_6}.$$

Confirm the presence of dextrose by the preparation of its osazone.

Maltose is not Hydrolysed by the Action of Invertase.—Prepare a solution of maltose of known concentration and proceed according to the second method described for the inversion of cane-sugar by yeast (see p. 84). Observe by means of the polarimeter that the maltose in the solution has not been hydrolysed by the invertase of the yeast.

Although this experiment demonstrates that the invertase of yeast has no action on maltose, since cane-sugar is inverted under similar conditions, it will be found later on when study-

ing yeast (p. 142), that the cells contain another enzyme, maltase, which under certain conditions hydrolyses maltose to dextrose.

Dextrin: Preparation of Dextrin.—The residue insoluble in alcohol which was left during the preparation of maltose (see p. 90) is employed.

Dissolve the residue in about half its weight of hot water, and add boiling alcohol of 90 per cent. strength to the solution until a permanent precipitate of dextrin is formed. Allow the solution to cool, pour off the mother-liquor, and extract the precipitate with boiling 80 per cent. alcohol to remove as much of the remaining maltose as possible. The precipitate now consists mainly of dextrin, but it will still retain some maltose. This can only be removed completely by a tedious process which includes fermentation and fractional precipitation; the process is too lengthy for the student to attempt.[1]

Specific Rotation of Dextrin.—This is usually considered to be $[\alpha]_D$ 202°.

Non-reducing Property of Dextrin.—Dextrin is generally considered to be a non-reducing carbohydrate.

Although it is usual to consider that the $[\alpha]_D$ of dextrin is 202° and that it has no reducing power, there is good reason to believe that stable dextrin possesses a feeble reducing power, or R, of about 5·5, compared with the reducing power of maltose

[1] See H. Brown and Millar, "The Stable Dextrin of Starch Transformations," *Journ. Chem. Soc.*, 1899, lxxv., p. 317.

reckoned as 100, and that its specific rotation is $[\alpha]_D$ 197-198°. These results have been obtained by H. Brown and Millar (see "The Stable Dextrin of Starch Transformations," *Journal of the Chemical Society*, 1899, lxxv., pp. 331 *et seq.*), who consider that the molecule of stable dextrin is probably represented empirically by the formula $\left.\begin{matrix}(C_6H_{10}O_5)_{39}\\ C_6H_{12}O_6\end{matrix}\right\}$, and that its reducing power is due to the single $C_6H_{12}O_6$, or glucose group, in the molecule. In analytical work connected with the carbo-hydrates the very small reducing power of dextrin is, however, generally ignored, and an $[\alpha]_D$ of 202° employed. This course is adopted in the following studies.

Preparation and Quantitative Examination of a Low Starch Conversion.—Starch paste when acted upon by diastase under the most favourable conditions for conversion is hydrolysed to a mixture of maltose and stable dextrin, the carbo-hydrates recently studied.

Prepare 100 c.c. of a 5 per cent. starch paste in a small beaker, cool to 55° (131° F.), and add 10 c.c. of malt extract (for preparation, see p. 87). Heat the solution in a water-bath to 55° (131° F.), for thirty minutes, when conversion will be complete if the malt extract used is sufficiently active.

While the starch conversion is in course of preparation, measure 10 c.c. of the malt extract used for the conversion into a 100 c.c. flask, dilute with water nearly to the mark, and heat the solution to 55° (131° F.) in the same water-bath as the starch conversion, and for a similar length of time. The solution is made in order to correct the specific

gravity, rotatory power, and reducing power of the starch conversion for the malt extract used in the conversion. It is treated in a similar manner to the starch conversion as it is liable to undergo change, which renders it desirable that the conditions of experiment should be similar in both cases.

Both the starch conversion and the check solution should now be boiled, and the reduced volume of each made up to 100 c.c. after cooling to 15·5° (60° F.). After filtration, determine the specific gravity, rotatory power and reducing power of the two solutions by the methods already described. (The reducing power is determined on 5 c.c. of each solution.) Now correct the different experimental values found for the starch conversion by subtracting the corresponding experimental values found for the check solution.

After making the required corrections, proceed to calculate the weight of starch conversion products present in 100 c.c. of the solution from the specific gravity of the solution by employing the appropriate solution factor for "Low Starch Conversions," which will be found on referring to Table II.

Then calculate the $[\alpha]_D$ of the conversion products in the usual manner from the weight of starch conversion products found and the observed rotatory power of the solution. The value found should approximate closely to $[\alpha]_D$ 150·3°, the value

for a complete conversion of starch into maltose and dextrin.

From the reducing power of the solution calculate the R, or percentage weight of maltose in the conversion products, which should approximate closely to 80·8, the value of R for a complete conversion.

Also calculate from the $[\alpha]_D$ found the percentage weight of maltose and dextrin present in the starch conversion, employing the values $[\alpha]_D$ 138° for maltose and $[\alpha]_D$ 202° for dextrin. Compare the amount of maltose found in this manner with the amount found by the copper reduction experiment. The two amounts should be in close agreement.

The transformation products in a starch conversion possessing an $[\alpha]_D$ 150·3° consist of :—

Maltose - - -	80·8	per cent.
Dextrin - - -	19·2	,, ,,
	100·0	

The optical activity of a starch transformation effected by unrestricted diastase falls rapidly from $[\alpha]_D$ 202°, representing the original soluble starch, to $[\alpha]_D$ 150·3° representing a (so-called) complete conversion, and when it reaches this stage the velocity of the transformation change is checked. $[\alpha]_D$ 150·3°, therefore, represents a well-defined point in the hydrolysis of starch.

An equation representing this change, and com-

monly called the "No. 8" equation, is given below :—

$$[5(C_{12}H_{20}O_{10})] + 4H_2O = 4C_{12}H_{22}O_{11} + C_{12}H_{20}O_{10}.$$
Starch. Maltose. Stable Dextrin.

It will be noticed that this equation represents that four-fifths of the starch molecule is converted into maltose and one-fifth into stable dextrin; but the amount of maltose in the *starch transformation products* is not of course four-fifths the weight of these products, owing to fixation of water during hydrolysis, hence the proportion 80·8 maltose to 19·2 dextrin, in the products of a complete conversion.

The equation representing the transformation of starch given above expresses the action in its simplest form, but the starch molecule is probably much larger than $[5(C_{12}H_{20}O_{10})]$.

Brown and Millar[1] bring forward evidence to show that the molecule of stable dextrin is $[20(C_{12}H_{20}O_{10})]$, and therefore that the molecule of soluble starch must be at least five times as large. According to this view, the conversion of soluble starch into maltose and dextrin may be represented as follows :—

$$\left\{\begin{array}{l}(C_{12}H_{20}O_{10})_{20}\cdot \\ (C_{12}H_{20}O_{10})_{20}\cdot \\ (C_{12}H_{20}O_{10})_{20}\cdot \\ (C_{12}H_{20}O_{10})_{20}\cdot \\ (C_{12}H_{20}O_{10})_{20}\cdot \end{array}\right. + 80H_2O = 80C_{12}H_{22}O_{11} + [20(C_{12}H_{20}O_{10})].$$
Soluble starch. Maltose. Stable dextrin.

The so-called "stable" dextrin of a starch conversion, although it strongly resists the action of diastase, is eventually hydrolysed to maltose and dextrose if the action of the diastase is very prolonged; but the velocity of the action is exceedingly

[1] See reference to Brown and Millar, p. 93.

slow as compared with the velocity of hydrolysis of starch to maltose and dextrin.

Influence of Heat on the Hydrolysis of Starch by Diastase.—When starch paste is acted on by diastase under the most favourable conditions for conversion it has been shown that the mixed products of hydrolysis possess an $[\alpha]_D$ 150·3°, corresponding to a mixture of 80·8 per cent. maltose and 19·2 per cent. dextrin. If, however, the action of diastase on starch is restricted by heat, the $[\alpha]_D$ of the transformation products does not fall so low as 150·3°, indicating the presence of less maltose and more dextrin in the conversion products.

Prepare 100 c.c. of a 5 per cent. starch paste, as in the previous experiment, but instead of cooling to 55° (131° F.), cool only to 68° (154·5° F.). Add 10 c.c. of malt extract previously heated to 68°, and keep the mixture in a water-bath at 68° for thirty minutes.

At the same time prepare a check solution of malt extract as in the previous experiment and submit it to the same conditions as the starch conversion.

Boil the starch conversion and check solution, cool and make up the reduced volume of each to 100 c.c. Filter, and determine, as in the previous experiment, the specific gravities, rotatory powers and reducing powers of the two solutions. Correct for the check solution, and after determining the solids present in the starch conversion by means

of the appropriate divisor, calculate the $[\alpha]_D$ of the transformation products.

From the observed $[\alpha]_D$ calculate the relative amounts of maltose and dextrin present, and check the result by means of a cupric oxide reducing determination.

It will be found that the $[\alpha]_D$, and therefore the apparent amount of dextrin, in this experiment is much higher than in the experiment conducted at the lower temperature of 55° (131° F.).

Experiment Showing that the Influence of Heat in restricting Starch Transformation is Due to Modification of the Diastase.—Digest about 50 c.c. of malt extract in a water bath at a temperature of 68° (154·5° F.) for thirty minutes. Note that the solution becomes turbid owing to matter being thrown out of solution. Cool the solution.

Prepare 100 c.c. of a 5 per cent. starch paste, cool to 55° (131° F.), as in the "low" starch conversion experiment, and add 10 c.c. of the previously heated malt extract. Proceed with the conversion exactly as in the "low" starch conversion experiment, making a check solution, as before. Determine the $[\alpha]_D$ of the solution, and compare with the $[\alpha]_D$ of the low starch transformation (p. 95). It will be observed that although both transformations have been carried on at the same temperature favouring complete hydrolysis (55°), the $[\alpha]_D$ of the transformation with malt extract previously heated indicates that its activity has been permanently restricted by this treatment.

Experiment Showing that although a Restricted Starch Transformation may be Represented as a Mixture of Maltose and Dextrin, much of the apparent Maltose cannot be Fermented by Ordinary Yeast and Exists as Malto-Dextrin.—This experi-

ment also introduces a method of analysing high starch transformation products.

Prepare 500 c.c. of a 5 per cent. starch paste, and after cooling it to 66° (151° F.), add 50 c.c. of malt extract previously heated to 66°. Keep the solution in a water-bath at 66° (151° F.) until it is found on testing that the iodine blue reaction of starch has disappeared but a *red erythro-dextrin reaction* remains. Then check the action of the diastase by rapidly heating the solution to the boiling point. Boil until the solution is reduced in volume to less than 500 c.c., and after cooling make up the volume again to 500 c.c. and filter.

At the same time prepare a check solution by diluting 25 c.c. of malt extract to 250 c.c. with water, and proceed as with the starch conversion.

Determine the specific gravities, reducing powers and rotatory powers of the two solutions. From the results obtained calculate :—

(1) The *total solids per* 100 c.c., using the appropriate divisor for high starch transformations.

(2) The $[\alpha]_D$ *of the solids of the starch conversion.*

(3) The *weight of maltose present in* 100 c.c. *of the conversion* on the assumption that the whole of the reducing power is due to maltose.

From the results of the experiment at this stage, it will be noticed that a high starch conversion has been obtained, and that the amount of the reducing matter of the transformation calculated as maltose is known ; but at present there

is no means of determining how much of the reducing matter is free maltose and how much is "apparent" maltose, existing as malto-dextrin or reducing dextrin.

Demonstrate qualitatively the presence of free maltose by heating 25 c.c. of the conversion for one hour with 1 grm. of phenyl-hydrazine dissolved in 1 grm. of 50 per cent. acetic acid. Note the formation of crystals of maltosazone when the solution cools. Malto-dextrins do not form crystalline osazones.

Determine the Amount of Free Maltose present in the Conversion.—Measure 350 c.c. of the conversion into a large flask, add about 3 grms. of washed pressed yeast, in order to ferment the maltose, and keep the solution at a temperature of about 26° (79° F.), until fermentation is complete. (This part of the experiment usually takes about forty-eight hours.) As a correction, ferment at the same time 100 c.c. of the check solution of diluted malt extract (see p. 100).

When fermentation is complete, boil the two solutions in order to expel the alcohol formed, and make them up to their original volumes with water and a little alumina cream.[1] Filter and determine the reducing power of each solution. Calculate the amount of "apparent" maltose present in 100 c.c. of the fermented conversion after correcting for the check solution.

Note that during fermentation of the starch conversion the free maltose it contained is de-

[1] The alumina cream is employed to aid clarification.

composed, and the "apparent" maltose existing as malto-dextrin is not fermented; hence the difference between the amounts of maltose found before and after fermentation represents the free maltose present in the original conversion. Calculate the amount of free maltose in 100 c.c. of the original conversion.

Demonstrate qualitatively that free maltose is no longer present in the fermented conversion by treating 25 c.c. with phenyl-hydrazine, as before. Note that no crystalline maltosazone is formed.

Calculate the Amount of "Apparent" Maltose in the Starch Conversion which exists as Malto-dextrin.—It has already been shown that the free maltose originally present in the conversion has been removed during fermentation, therefore the reducing power of the fermented conversion, after correcting for the malt extract used, is due to "apparent" maltose existing as malto-dextrin. Calculate the amount of "apparent" maltose found in 100 c.c. of the conversion.

Determine the Amount of "Apparent" Dextrin existing as Malto-dextrin. — It has already been shown that the stable dextrin of a low starch conversion is not hydrolysed (or is only hydrolysed with extreme slowness) by malt extract (p. 96), but a marked property of the "apparent" dextrin of a high starch transformation is the readiness with which it is hydrolysed to maltose by unrestricted diastase; or, expressed in another

manner, a high starch transformation produced by restricted diastase is readily hydrolysed to the low starch transformation (No. 8 equation), containing maltose and stable dextrin only, by the further action of active diastase.

The following example explains the general character of the action during a restricted conversion :—

$$\overbrace{100(C_{12}H_{20}O_{10})}^{\text{Soluble Starch.}} + 60\ H_2O = \overbrace{40C_{12}H_{22}O_{11}}^{\text{Maltose.}}$$

$$+ \overbrace{20\begin{cases} C_{12}H_{22}O_{11} \\ C_{12}H_{20}O_{10} \end{cases}}^{\text{Malto-dextrin.}} + \overbrace{20(C_{12}H_{20}O_{10})}^{\text{Stable Dextrin.}}.$$

On the further addition of unrestricted diastase to the restricted conversion the malto-dextrin only is acted on :—

$$\overbrace{20\begin{cases} C_{12}H_{22}O_{11} \\ C_{12}H_{20}O_{10} \end{cases}}^{\text{Malto-dextrin.}} + 20H_2O = \overbrace{40C_{12}H_{22}O_{11}}^{\text{Maltose.}}.$$

As a final result, therefore, the original restricted conversion is brought down to the low starch transformation, $[\alpha]_D$ 150·3°, represented by :—

$$\underset{\text{Maltose.}}{80C_{12}H_{22}O_{11}} + \underset{\text{Stable Dextrin.}}{20(C_{12}H_{20}O_{10})}$$

To 200 c.c. of the fermented conversion, add 10 c.c. of malt extract and digest the mixture in a water-bath at 55° (131° F.) for one hour. The malto-dextrins present will thus be converted into free maltose.

Make a check solution by adding 5 c.c. of malt extract to 100 c.c. of water, and treat in a similar manner to the conversion.

Boil, cool, and make up the two solutions to 200 c.c. and 100 c.c. respectively with water.

Filter and determine the reducing power of both solutions. The reducing power of the degraded solution corrected for the malt extract used, less the reducing power of the solution previous to degrading (not corrected for malt extract), is due to maltose derived from the "apparent" dextrin which existed as malto-dextrin.

Calculate the amount per 100 c.c., first as maltose, and afterwards as the equivalent amount of dextrin, according to the equation :—

$$\underset{\text{Dextrin.}}{C_{12}H_{20}O_{10}} + H_2O = \underset{\text{Maltose.}}{C_{12}H_{22}O_{11}}$$

Constitution of the Malto-Dextrin Found.—As the amounts of "apparent" maltose and dextrin existing as malto-dextrin in 100 c.c. of the starch conversion have now been determined, it is now possible to express in a simple form the proportion in which they appear to be combined (*e.g.*, 1·5 maltose, 1 dextrin). The proportion is sometimes regarded as indicating the type of malto-dextrin found.

Note that the proportion of "apparent" maltose to "apparent" dextrin found by the above method of experiment may only express the proportion of a mixture of malto-dextrins of varying reducing power.

Determine the Amount of Stable Dextrin present in the Conversion.—Measure 150 c.c. of the degraded conversion (see above) into a flask, add about 1·5 grms. of washed pressed yeast, and allow the solution to ferment at a temperature of 26° (79° F.), until the whole of the maltose present is decomposed. Boil

the solution to expel the alcohol formed, cool and make up to the original volume of 150 c.c. Filter and determine the rotatory power of the solution.

Measure 100 c.c. of the fermented solution into a small flask, add 5 c.c. of malt extract and about 1 grm. of pressed yeast, and keep the flask at a temperature of 26° (79° F.) until all signs of fermentation have disappeared. Also make a check solution of 100 c.c. water, 5 c.c. malt extract and 1 grm. pressed yeast, and ferment as above.

Note that in this experiment stable dextrin, which is not fermented by yeast under ordinary conditions, has been submitted to the joint action of diastase and yeast. Neither of these agents alone is able to hydrolyse stable dextrin, the resisting power of which is however overcome by the joint action of the two, with the result that the dextrin is fermented.

Boil the fermented conversion and the check solution to expel the alcohol formed, cool and make both up to the original volume of 100 c.c. Filter, determine the rotatory power of both solutions, and correct the starch conversion result for the malt extract used. The stable dextrin may now be calculated from the loss in rotatory power of the conversion during fermentation in the presence of malt extract.

When the analysis of the high starch transformation is completed, express the results as amounts of maltose, malto-dextrin, and stable dextrin in 100 c.c. of the conversion.

PART III.

Studies Bearing Directly on the Technology of Brewing.

A Study of the Influence of the Mashing Temperature on the Optical Activity of Malt Worts.—Take two beakers, each containing 250 c.c. of water heated to 148° F. and 161° F. respectively, and mash into each beaker 50 grms. of ground pale malt (from the same sample). The mashes should then have "initial" heats of 144° F. and 157° F., and they must be kept at these temperatures for one hour by being placed in separate water baths heated to the required temperatures. Cool, filter, and determine the specific gravities and rotatory powers of the two worts. Use the 3·86 factor to determine the weight of solids present, and calculate the $[\alpha]_D$ of the two worts.

Note that the specific rotation of the wort from the high temperature mash is higher than that from the low, indicating the presence of more dextrin in the former than in the latter. The reason for this will be found on considering the results of the experiments already made with high and low starch transformations.

Measure 100 c.c. of each of the two worts into separate flasks, boil for twenty minutes to destroy the diastase present, cool, and make up the volumes again to 100 c.c. Add about 0·5 grm. of pressed

yeast to each solution and ferment in a warm place. When fermentation is complete, filter, and determine the specific gravity or "attenuation" of the fermented worts. Note that the specific gravity increases with the mashing temperature and $[\alpha]_D$ of the original wort, indicating that the high temperature mash contains a larger amount of unfermentable matter than the low temperature one. Consider this in the light of the experiment made with a high starch conversion.

The divisor 3·86 is employed in the above experiments as it is customary to use it in determinations of the $[\alpha]_D$ of brewery worts. The divisor (3·86) was originally obtained from experiments with cane sugar and the divisor 4·00 is more accurate for malt wort solids, but custom at present decides in favour of 3·86, and it is employed here for this reason. As the chief value of determinations of the $[\alpha]_D$ of worts lies in their use for purposes of comparison, the employment of the incorrect 3·86 factor is of little moment so long as its use is indicated in the proper manner, *e.g.*, $[\alpha]_{D3\cdot86}$.

The student is often surprised to find, on first determining the $[\alpha]_D$ of a malt wort, that it is much lower than the $[\alpha]_D$ of a low starch transformation (150·3°), although it represents a conversion restricted by heat. The low $[\alpha]_D$ is occasioned by the presence in malt wort of bodies other than starch transformation products which, as a whole, possess little or no rotatory power.

Experiments on the Fermentation of Boiled and Unboiled Worts, Illustrating the Different Methods of the Brewer and the Distiller or Vinegar Maker. —Mash 100 grms. of ground pale malt in a beaker with 500 c.c. of water at a temperature of 154° F. and keep the mash at a temperature of 150° F. for

one hour. Filter, and measure out two samples of 100 c.c. of the wort into two flasks. Boil one sample for twenty minutes to destroy the activity of the diastase present; cool, and make up to the original volume of 100 c.c. Add 0·5 grm. of pressed yeast to both the boiled and unboiled worts, and keep them at a temperature of about 26° (79° F.) until fermentation is complete. In the case of the unboiled wort the fermentation may take three or four days. Filter the fermented worts and determine their specific gravities ("attenuations").

Note that the specific gravity of the boiled and fermented wort is much higher than that of the unboiled one. It has already been shown by a previous experiment (p. 105) that the malto-dextrins and stable dextrin are fermented by yeast in the presence of active diastase; during the fermentation of the unboiled wort this action has gone on, leading to the fermentation of a larger proportion of malt wort solids than in the boiled wort in which the malto-dextrins and stable dextrin remain unfermented. Fermentation of an unboiled wort, therefore, tends to yield the highest possible amount of alcohol—the object of the distiller and vinegar maker. Fermentation of a boiled wort, on the other hand, yields a smaller amount of alcohol, but a liquid containing malto-dextrin and dextrin—the object of the brewer.

A Study of the Rise in Temperature observed when mixing Dry Malt with Cold Water.—Weigh

25 grms. of finely ground, well-dried malt in a dry 100 c.c. beaker, and cover the beaker with a watch-glass. At the same time measure 60 c.c. of cold water into another beaker of similar size. Place the two beakers side by side, and allow them to remain so for about an hour in order that the contents of both may attain the temperature of the laboratory.

Note that the quantities of malt and water taken are in the same proportion as those commonly employed in technical mashing operations, and that the malt and the water before mixing are at the same temperature.

Ascertain the temperature of the water with a chemical thermometer, and then pour the water into the beaker containing the dry malt and mix thoroughly, using the thermometer for stirring. As the mixing proceeds notice the rise of temperature taking place in the mash, and continue stirring the mash until the maximum temperature is reached, which will probably be observed in two or three minutes.

If the sample of malt experimented on is very dry, the observed rise in temperature may be as much as 8° F.; if the malt is very slack the rise may possibly not exceed 4° F. The rise in temperature is due to a loose molecular combination of the water with the starch and other constituents of the malt. Note that the evolution of heat observed in a cold mash must take place equally

during the preparation of a hot mash in the brewery, and therefore that the relative dryness of malts must exert a marked influence on the "initial" heats obtained in brewery mashing operations.

Analysis of a Fermented Wort or Beer.—The operations involved in an analysis of a fermented wort or beer may be subdivided into two parts :—

1. A determination of the "original gravity," and the amount of alcohol present in the sample.

2. An analysis of the unfermented matter in the sample.

1. The term "original gravity" as applied to a fermented wort or beer means the specific gravity of the original wort before fermentation.

The method very generally employed in this country for the purpose of determining the original gravity of fermented liquids is one originated by the excise authorities and recognised by the excise laws. The method is based on the following considerations :—

During the fermentation of a wort the fermentable matter it contains is decomposed into alcohol and carbon dioxide, the former remaining in the fermented liquid, the latter being evolved as a gas. As a consequence, the specific gravity (or original gravity) of the liquid diminishes during fermentation, for not only does the liquid lose matter which originally contributed towards its weight, but this

matter is replaced by alcohol—a liquid having a less density than water—which further tends to diminish the specific gravity of the fermented wort. The specific gravity of a fermented wort represents therefore the weight in solution of neither the unfermented matter present nor the alcohol, for, since the density of the one is greater than water and that of the other is less, the joint influence of the two tends to obscure the weight of both. If, however, the alcohol in a measured volume of the solution is separated by distillation from the non-fermented matter and the volumes of both the distillate and the non-volatile residue are made up to the original volume, then the specific gravity of the distillate expresses the weight in solution of the alcohol present, and that of the residue expresses the weight in solution of the unfermented matter present.

It will now be evident that if the solution weight of the fermented matter can be determined from the weight of the alcohol, this weight added to the solution weight of the unfermented matter found will express the original gravity of the wort before fermentation. Although the solution weight of the fermented matter cannot be determined satisfactorily from the alcohol by direct calculation, it may be arrived at by means of a special table based on the results of a series of direct experiments on fermenting wort in which the solution weights of fermented matter corres-

ponding to varying amounts of alcohol have been determined (see Table V.).

Determination of the Original Gravity of a Fermented Wort or Beer.—If the beer contains carbon dioxide, remove it by agitation or filtration. Measure 100 c.c. of the beer in a graduated flask at the standard temperature of 60° F. Pour the measured volume into the distilling flask of an original gravity distilling apparatus, and afterwards rinse the measure with about 40 c.c. of water and transfer the washings to the distilling flask. Distil the fermented wort, collecting the distillate in a 100 c.c. measure until 80 to 90 c.c. have passed over, when the whole of the alcohol will be present in the distillate. Make up the distillate with water to 100 c.c. Cool the residue in the distilling flask and transfer it together with the washings of the flask to a 100 c.c. measure, and make up the volume with water to 100 c.c. Determine accurately the specific gravity of the two solutions at 60° F.

The specific gravity of the residue represents the weight in solution of the unfermentable matter in the original wort. The specific gravity of the distillate represents the fermented matter expressed as the weight of a mixture of alcohol and water. In order to find the amount of fermented matter, subtract the specific gravity of the alcoholic distillate from 1,000 and the difference is the so-called "spirit indication" number. Refer to Table V., and find by means of the "spirit indication" num-

ber the number of "degrees" of specific gravity lost during fermentation. Add this number to the specific gravity of the unfermented matter, and the sum is supposed to represent the original gravity of the fermented wort.

It should be noted, however, that this method requires correction if the acidity of the beer experimented on exceeds 0·1 per cent. calculated as acetic acid. It is assumed, somewhat incorrectly, that any excess of acidity over 0·1 per cent. is formed at the expense of the alcohol in the beer, and that consequently the original gravity will appear too low if the excess of acid is not allowed for.

To correct for the acidity of a beer, determine the amount of acid present by titration with decinormal ammonia solution and litmus-paper, and calculate as acetic acid. Subtract 0·1 per cent., which is allowed for in the ordinary spirit indication table, and refer the difference to Table VI., which has been constructed for the purpose of indicating the correction due to the excess acid found. Add the number found to the spirit indication figure, and proceed with the calculation as before.

The method of determining original gravities just described, although adopted officially as the standard method for this country, is not very accurate. When it is employed for the purpose of ascertaining the original gravities of finished beers of medium gravity the results obtained are usually about two degrees too low, and a correction for

the error should be employed. With fermenting worts containing yeast and little alcohol the results are, however, fairly accurate.

Determination of the Amount of Alcohol in a Fermented Wort or Beer. — Refer the specific gravity of the distillate (p. 112) to alcohol tables showing the weight of alcohol corresponding to varying specific gravities of aqueous spirit, and from the weight found calculate the weight of alcohol present in 100 c.c. of the original beer.

2. **Analysis of the Matter remaining Unfermented in a Fermented Wort or Beer.** — The analysis is conducted in a similar manner to that of a high starch conversion (p. 99), but is subject to correction for the optical activity and reducing power of certain constituents other than maltose, malto-dextrin and dextrin.

Boil 500 c.c. of the fermented wort until its volume is reduced to about one-half in order to expel the alcohol present. Cool, and make up the residue to its original volume with water. Determine the specific gravity of the solution, and calculate the grms. of solid matter present in 100 c.c., using the 4·0 factor.

Determine the rotatory power (in 100 mm.) and reducing power of the solution, and express the reducing power as grms. maltose in 100 c.c.

Determine the Amount of Free Maltose in the Solution.—Ferment 250 c.c. of the solution with 2 grms. of pressed yeast. Boil the solution to

expel alcohol, and make up to the original volume with water and a little alumina cream. Filter, and determine the reducing power of the solution. Calculate the reducing power as maltose, and express as grms. maltose in 100 c.c.

The difference in the amount of maltose found in 100 c.c. of the solution before and after fermentation represents the free maltose in 100 c.c. of the solution. (Note that possibly some of the maltose found may have existed as low type, easily fermentable, malto-dextrin.)

Determine the Amount of "Apparent" Maltose combined as Malto-Dextrin.—The reducing power of the fermented solution, *less the correction for the reducing bodies other than sugar*, is due to "apparent" maltose combined as malto-dextrin. Express as grms. combined maltose in 100 c.c. (Note that the correction for the reducing bodies other than combined maltose is determined at a later stage of the experiment.)

Determine the Amount of "Apparent" Dextrin combined as Malto-Dextrin.—Treat 200 c.c. of the fermented solution with 10 c.c. malt extract at 50° (122° F.) for one hour. Also make a check solution of 5 c.c. malt extract with 100 c.c. water, and treat in a similar manner to the fermented solution. Proceed as in the analysis of a high starch conversion (see p. 102), and calculate the amount of "apparent" dextrin as malto-dextrin in 100 c.c. of the solution.

Also calculate the constitution of the malto-dextrin found (see p. 104).

Determine the Amount of Stable Dextrin.—Ferment 150 c.c. of the degraded conversion in order to remove the free maltose present; boil, cool and make up to the original volume.

Determine the rotatory power of the solution in a 100 mm. tube.

To 100 c.c. of the solution, add 5 c.c. of malt extract, and ferment with about 1 grm. of freshly pressed yeast to decompose the stable dextrin present. Also prepare a check solution with 5 c.c. of malt extract in 100 c.c. water, and ferment as above. When fermentation is complete in both solutions, boil to expel the alcohol present, and make up in each case to 100 c.c. after adding a little alumina cream. Filter, and determine the rotatory powers (in 100 mm.) and reducing powers of both solutions. Correct the rotatory power and reducing power of the fermented solution for those of the malt extract used.

Note that the remaining reducing power is due to constituents other than maltose and must be used as a correction in the determination of the "apparent" maltose combined as malto-dextrin (see p. 102).

The stable dextrin may now be determined from the difference in rotatory power of the solution before and after fermentation in the presence of malt extract.

Express the results of the analysis in grms. per 100 c.c., the difference between the weight of total solids and the sum of the weights of maltose, malto-dextrin and stable dextrin found being expressed as "undetermined matter".

PART IV.

Analysis of Brewing Sugars.

The most satisfactory method at present available for the purpose of analysing brewing sugars is one suggested by Dr. G. H. Morris (*Journal of the Federated Institutes of Brewing*, 1898, vol. iv., p. 162). The method is described below, but the student is recommended to consult Dr. Morris's original paper for full information.

Analysis of Invert-Sugars.—The analysis includes determinations of the ash, albuminoids, water, dextrose, levulose, cane-sugar, unfermentable matter and the brewer's extract.

Ash.—Weigh out about 5 grms. of the sample in a 50 c.c. platinum dish, add 2 c.c. of strong sulphuric acid, and heat gently over a Bunsen burner; when the first violent intumescence has subsided, place the dish with its charred contents in a muffle furnace, and heat until the residue is quite white. Cool in a desiccator, and weigh. Calculate the percentage amount of sulphated ash found.

Albuminoids.—Weigh about 2 grms. of the sample in a 100 c.c. Jena glass beaker or flask, and treat with 20 c.c. concentrated sulphuric acid. Add 5 grms. of potassium sulphate and heat the mixture, at first very gently, and afterwards strongly, until decolourisation is complete. Proceed subsequently to determine the nitrogen present according to the usual Kjeldahl method, and calculate the percentage of albuminoids found, using the 6·3 factor (see p. 59).

Brewer's Extract and Water.—Dissolve 25 grms. of the sugar in boiling water, cool the solution and make up to 250 c.c. at 15·5° (60° F.). Determine the specific gravity of the 10 per cent. solution thus obtained.

Calculate the Brewer's Extract.—The specific gravity, by the removal of the decimal point one place to the left, indicates the weight of 100 c.c. of the solution, and the excess weight of this volume above 100 grms. indicates the weight in solution of 10 grms. of the sugar.

From the amount found calculate the weight in solution of 224 grms. of the sugar, and express the result as pounds of brewer's extract derived from 224 lb. (or 2 cwt.) of the sugar.

Calculate the Water.—Determine the solid matter present in 100 c.c. of the 10 per cent. solution from its specific gravity by means of the 3·86 divisor. The amount of solids found requires, however, to be corrected for the high solution

density of the ash present, for the ash of sugars has a solution density rather more than twice that of the carbo-hydrates, *i.e.*, the divisor for the ash is about 8. The most convenient way of correcting for the ash is to multiply the solid matter found in 100 c.c. by 10, in order to convert it into a percentage, and then to deduct from the amount the percentage of ash already found. The percentage weight of solid matter obtained in this manner, deducted from 100, gives the percentage weight of water in the sugar.

Reducing Sugars.—Dilute 10 c.c. of the 10 per cent. sugar solution used in the above experiment to 50 c.c., and take 10 c.c. of the diluted solution for an estimation of the reducing power, the operation being conducted in accordance with the usual standard conditions (see p. 79). In this way the amount of copper reduced by 0·2 grms. of the sugar is obtained. Calculate from the amount found the quantity reduced by 100 grms. of the sugar by multiplying by 500. This value then requires correcting for the reducing power possessed by the unfermentable residue of the sugar, which is subsequently determined ; it then expresses the reducing power of the dextrose and levulose in 100 grms. of the sugar.

The rotatory power of the 10 per cent. sugar solution is then determined in the half-shadow instrument at a temperature of 20° (68° F.), and is expressed in *divisions*, care being taken that the

bi-rotation of the sugar solution has been previously destroyed. But the value found requires correcting for the opticity of the cane-sugar and that of the unfermentable matter present in the sugar, which are subsequently determined. The corrected value in divisions of the half-shadow instrument is then employed to calculate the $[a]_D$ of the sugar on the basis that the solution contains 10 grms. in 100 c.c.

The percentages of the dextrose and levulose in the sugar are now calculated by means of a pair of simultaneous equations in which :—

xD = the gram. value of dextrose expressed as Cu.
xL = the gram. value of levulose expressed as Cu.
a = the Cu. reduced by 100 grms. of the sugar.
b = the specific rotatory power ($[a]_D$).

The equations then are :—

$$x\mathrm{D} + x\mathrm{L} = a$$
$$[a]_D\mathrm{D} + [a]_D\mathrm{L} = b \times 100.$$

Cane-Sugar.—In order to determine the amount of uninverted cane-sugar, if any, present in the invert-sugar, 50 c.c. of the 10 per cent. solution are measured into a double-marked 50 to 55 c.c. flask at 15·5° (60° F.), and digested with 0·5 grm. of pressed yeast for six hours at 50° (122° F.). The solution is then cooled to 15·5° (60° F.), a little alumina cream added, made up to 55 c.c. and filtered. The rotation of the solution in the 200 mm. tube is then determined at 20° (68° F.), in divisions of the half-shadow polarimeter. The

reading is increased in the proportion of 55 to 50 in order to correct for the dilution of the solution, and is then subtracted from the original reading of the solution (in a 200 mm. tube) before inversion. The difference, divided by 5·02, gives the cane-sugar; and this, multiplied by 10, gives the percentage of cane-sugar in the sugar analysed.

The divisor 5·02 used in the above experiment is the number of divisions of the half-shadow polarimeter which a solution of 1 grm. of cane-sugar in 100 c.c. of water, read in a 200 mm. tube, loses on being converted into invert-sugar.

Unfermentable Matter.—50 c.c. of the 10 per cent. solution of the sugar are placed in a 100 c.c. flask, and sterilised by boiling for a few minutes. After cooling, 2 grms. of washed and pressed yeast are added, and the mixture is allowed to ferment at about 24° (75° F.). When fermentation is complete, which will probably be in about forty-eight hours, a little alumina is added to the fermented liquid and its volume is made up to 100 c.c. at 15·5° (60° F.).

After filtration, the reducing power is determined on 25 c.c. of the solution, and the rotation read in the 200 mm. tube. The values so obtained are then expressed in the same manner as those of the original solution for the purpose of correcting the reducing sugar values as already described (see p. 119).

The percentage weight of the unfermentable matter in the invert-sugar is usually obtained by

difference; *i.e.*, by subtracting from 100 the total weight of the sugars, ash, albuminoids and water found.

The following example illustrates the method of analysing an invert-sugar described above :—

Analysis of an Invert-Sugar.

Ash.—A direct estimation showed the presence of 1·54 per cent. of ash.

Albuminoids.—A determination by Kjeldahl's method, as described (p. 118), gave 0·24 per cent. of albuminoids.

Water.—The specific gravity of the 10 per cent. solution of the sugar was 1031·06, which indicates, by the use of 3·86 as a divisor, the presence of 8·046 grms. of solids per 100 c.c. Multiplying this by 10 gives 80·46 as the percentage weight of solid matter in the sugar. But this amount has to be corrected for the solution density of the ash (see p. 119). To do so, the percentage weight found, 1·54, is subtracted from 80·46, leaving 78·92 as the corrected percentage weight of solids in the sugar. Then 100 - 78·92 = 21·08, the percentage weight of water in the sugar.

Cane-Sugar.—The 10 per cent. sugar solution before inversion gave a reading of - 5·25 divisions of the half-shadow polarimeter in the 200 mm. tube. After inversion and treatment as described above, the reading corrected for dilution was - 5·77 divisions in the 200 mm. tube. Hence the difference in the readings before and after inversion is 0·52

division, due to the conversion of cane-sugar into invert-sugar. Then—

$$\frac{0{\cdot}52 \times 10}{5{\cdot}02} = 1{\cdot}03 \text{ per cent. cane-sugar.}^{1}$$

Unfermentable Residue.—After fermentation of the 10 per cent. sugar solution and treatment as described above (p. 121), the reading in the 200 mm. tube was − 0·10 division, and 25 c.c. reduced 0·032 grm. of Cu.

Reducing Sugars.—Ten c.c. of the 10 per cent. solution of the invert-sugar were diluted to 50 c.c., and 10 c.c. of the diluted solution reduced 0·2759 grm. Cu. The rotation in the 200 mm. tube of the 10 per cent. solution was − 5·25 divisions.

The reduced copper, 0·2759 grm., multiplied by 500 gives the amount of copper which would be reduced by 100 grms. of the original invert-sugar, *viz.*, 137·95 grms. Cu. But this amount has to be corrected for the amount of copper reduced by the unfermentable residue. Twenty-five c.c. of the half-diluted fermented solution reduced 0·032 grm. Cu. Therefore 0·032 × 80 = 2·56 grms. Cu. reduced by the unfermentable matter in 100 grms. of the sugar.

Hence 137·95 − 2·56 = 135·39 grms. Cu. reduced by the dextrose and levulose in 100 grms. of the invert-sugar.

The observed rotatory power of the 10 per cent. solution in the 200 mm. tube has now to be corrected

[1] See p. 121.

for the rotatory power of the cane-sugar and the unfermentable matter present. 0·103 grm. of cane-sugar were found in 100 c.c. of the solution, and the calculated rotation for this amount in the 200 mm. tube is 0·39 division; the corrected reading found for the unfermentable residue was − 0·2 division in the 200 mm. tube. Hence the rotation due to the cane-sugar is added to, and that of the unfermentable residue is subtracted from, the original reading (− 5·25), leaving a corrected reading of − 5·44 divisions, which gives on calculation an $[\alpha]_D$ − 9·44°.

On referring to Table III., it is found that with a reduction of 0·2759 grm. of Cu. the grm. value of dextrose is 1·975, and that of levulose 1·814; the simultaneous equations for calculating the percentages of dextrose and levulose in the original invert-sugar then become :—

$$\left.\begin{array}{l} 1{\cdot}975\ D + 1{\cdot}814\ L = 135{\cdot}39 \\ 52{\cdot}8\ D + (-92{\cdot}0\ L) = -9{\cdot}44 \times 100 \end{array}\right\} = \left\{\begin{array}{l} \text{Dextrose, } 38{\cdot}65 \text{ p.c.} \\ \text{Levulose, } 32{\cdot}44 \text{ ,,} \end{array}\right.$$

The result of the complete analysis of the invert-sugar is therefore :—

Dextrose	38·65	per cent.
Levulose	32·44	,,
Cane-sugar	1·03	,,
Ash	1·54	,,
Albuminoids	0·24	,,
Water	21·08	,,
Unfermentable matter (by difference)	5·02	,,
	100·00	,,

Analysis of Glucose or Starch Sugar.—The analysis is carried out in the same manner as the analysis of invert-sugar described above, but the determination of the cane-sugar is omitted as this sugar does not occur in sugars prepared from starch. When calculating the reducing sugars present the constant for maltose is substituted for that of levulose.

Thus, in an analysis of a glucose, in which 1·45 per cent. of ash, 0·97 per cent. of albuminoids and 15·70 per cent. of water were obtained, the Cu. reduced by 100 grms. of the glucose was found to be, after due correction, 121·97 grms., and the corrected opticity $[\alpha]_D$ 41·35. The simultaneous equations for calculating the percentage amounts of dextrose and maltose then become :—

$$\left.\begin{aligned} 1{\cdot}979\ D + 1{\cdot}089\ M &= 121{\cdot}97 \\ 52{\cdot}8\ D + 138{\cdot}0\ M &= 41{\cdot}35 \times 100 \end{aligned}\right\} = \begin{cases} \text{Dextrose, } 57{\cdot}16 \text{ p.c.} \\ \text{Maltose, } \ 8{\cdot}09 \ \text{,,} \end{cases}$$

The result of the complete analysis of the glucose is therefore :—

Dextrose - - - - -	57·16 per cent.
Maltose - - - - - -	8·09 ,,
Ash - - - - - -	1·45 ,,
Albuminoids - - - - -	0·97 ,,
Water - - - - - -	15·70 ,,
Unfermentable matter (by difference)	16·63 ,,
	100·00

SECTION III.

FERMENTATION.

INTRODUCTION.

The following studies are divided into two parts—one concerned more especially with the physiological aspect of fermentation, and the other with the morphology and life history of the more important micro-organisms of fermentation and with the special methods employed in their examination; but the division is a somewhat arbitrary one, and many points relating to the first part are of necessity studied in the second.

In the earlier courses of study described in this book it was found necessary to enter into detail regarding much of the experimental work, as there was no text-book covering the whole of the required ground to which the student could be referred for information; but there are several text-books on fermentation available, and in the following studies it is proposed to refer to these whenever possible in order to avoid enlarging the present work unnecessarily. For this reason the space devoted to the study of fermentation may

appear limited when compared to that devoted to other studies, but the student must not be misled by this and underrate the length of time required for his fermentation work, for he will perhaps find it necessary to devote more time to it than to any of his previous studies.

PART I.

The Physiological Aspect of Fermentation.

It is not practicable for a student in the time usually at his disposal to study the physiological aspect of fermentation in the laboratory in anything like a systematic manner, for the subject is a very large one, and experiments connected with it take up much time. The following short course of experiments must therefore be regarded as merely an introduction to the experimental side of the subject. It is expected that the student will have become familiar by means of lectures and books with the modern views of fermentation and the life history of fermentation organisms before commencing the experiments described; following on this, the short course of experimental work will then put him in touch practically with the general bearings of the subject.

Determination of the Amount of Alcohol and Carbon Dioxide produced during the Fermentation of Sugar by Yeast.—Prepare a small, light flask of about 200 c.c. capacity with a perforated rubber

stopper furnished with a chloride of calcium tube and a side tube as represented in the annexed figure, and provide the side tube with a removable stopper made with rubber tubing and a small piece of glass rod. When constructing the apparatus, note that it must be made of such a size as to admit of it being placed on the pan of a chemical balance. Weigh the apparatus accurately on the balance, and then introduce about 8 grms. of powdered pure dry cane-sugar into the flask and re-weigh to ascertain the weight of sugar taken. Introduce about 90 c.c. of yeast water (for preparation of yeast water, see p. 145), and shake the flask gently until the sugar is dissolved. Add to the solution about 0·5 grm. of pressed yeast rendered liquid with a little yeast water, and re-weigh the apparatus immediately. After weighing, place the apparatus in an incubator kept at a temperature of about 24° (75° F.). Active fermentation will commence in a few hours, and it is advisable in the earlier stage of fermentation to note if there is any danger of the frothy "head" rising into the neck of the flask. If it appears likely for this to occur the apparatus should be removed to a cooler place

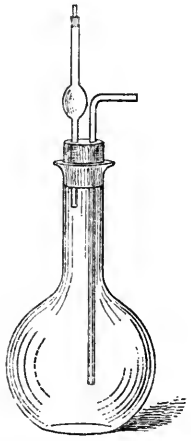


FIG. 19.—Apparatus for the Determination of Carbon Dioxide and Alcohol Generated during Alcoholic Fermentation.

until the more active stage of fermentation is past. When fermentation of the sugar in the flask is complete, which is usually the case in about sixty hours, remove the cap from the side tube and attach some form of aspirator to the drying tube of the apparatus by means of rubber tubing, and draw a current of air slowly through the liquid in the flask in order to expel the carbon dioxide present. Disconnect the aspirator and weigh the apparatus. Again draw more air through the flask and again weigh, and, if required, repeat the operation until a constant weight is obtained, showing that all the carbon dioxide has been removed. When this operation is complete, the difference between the weight of the apparatus before and after fermentation represents the weight of carbon dioxide liberated during the fermentation of the cane-sugar originally taken.

In order to determine the amount of alcohol formed during fermentation, transfer the fermented solution and rinsings of the flask to the flask of an original gravity still, and distil over about 90 c.c. into a 100 c.c. flask. Make up the volume of the distillate to 100 c.c. at 15·5° and determine its specific gravity accurately. Refer the specific gravity found to a table[1] giving the densities of mixtures of alcohol and water, and calculate the weight of alcohol present in the distillate. The weight found represents the amount of alcohol

[1] See Hehner's alcohol tables.

formed during fermentation of the cane-sugar originally taken.

Calculate the percentage weights of carbon dioxide and alcohol obtained from cane-sugar, and compare with those obtained by Pasteur :—

Carbon dioxide - - -	49·42 per cent.
Alcohol - - - -	51·11 ,,

The amounts found by the method of experiment described above usually agree with Pasteur's figures within 0·5 per cent.

The student should now calculate the percentage amounts of carbon dioxide and alcohol which would be obtained from cane-sugar if it fermented completely according to the equation :—

$$C_{12}H_{22}O_{11} + H_2O = 4CO_2 + 4C_2H_5HO.$$

Determination of Glycerin as a Secondary Product of Alcoholic Fermentation.—The residual liquid obtained in the previous experiment after removal of the alcohol may be used for this determination, but the result obtained with such a small volume of liquid is not usually very satisfactory. It is better to ferment 25 grms. of cane-sugar dissolved in about 250 c.c. of yeast water for the special purpose of the determination.

Evaporate the fermented solution in a porcelain dish on a water bath to a volume of about 50 c.c. Add 20 grms. of powdered animal charcoal to the liquid, mix well and evaporate the mixture to dryness at a temperature of about 70° C. Transfer the whole of the dried residue to a mortar, add 20 grms. of quicklime, and triturate the mixture until it is reduced to a fine grey powder. The object of adding the lime is to thoroughly dry the mixture, and also to neutralise and render insoluble the

succinic acid present. Transfer the whole of the powder to a small dry flask, and add about 80 c.c. of specially dried ethyl acetate free from alcohol. Shake the mixture well for several minutes, and after allowing the precipitate to settle, decant off the clear supernatant liquid and pass it through a filter. Add another 80 c.c. of ethyl acetate to the residue, repeat the shaking and transfer the whole of the mixture to the filter. After the liquid has drained through, wash the precipitate with a little more ethyl acetate. The mixed filtrates now contain all the glycerin. Transfer to a small flask and distil off the ethyl acetate by heating in a water bath until about 30 c.c. of solution are left. Transfer to a small tared porcelain dish, and evaporate in an air bath at 60° C., until the weight is constant. The residue, glycerin, should be of a light yellow colour, and possess a pure sweet taste.

Calculate the percentage weight of glycerin formed during the fermentation of cane-sugar.

Chemical Composition of Yeast. — Freshly pressed brewer's yeast, if it is sufficiently dry to crumble, may be used for the following experiments; but if ordinary pressed yeast in good condition is not obtainable, liquid yeast may be strained on a cloth filter, and the filter and pasty yeast placed in the folds of a strong cloth and submitted to pressure by twisting the ends of the cloth in opposite directions with considerable force. By this means yeast may be obtained as a dry crumbly mass. Pressed yeast may be regarded as a mass of yeast cells separated from the liquid in which they were originally contained.

1. *Determination of Water in Yeast.*—Weigh about 0·75 grm. of finely crumbled pressed yeast in a drying tube. Transfer the tube to a drying

oven, raise the temperature very slowly to 100°, and continue drying at this temperature until the weight is constant. From the weights obtained before and after drying calculate the percentage weight of water in the yeast.

2. *Determination of the Ash of Yeast.*—Weigh 10 grms. of pressed finely crumbled yeast in a platinum dish, and dry it slowly on a water-bath. When dry, ignite the yeast gently with a Bunsen flame until the residue is thoroughly carbonised, and afterwards ignite more strongly in a muffle furnace until a very light grey ash is obtained. Weigh, and calculate the percentage weight of ash in the pressed yeast taken. Also calculate the percentage weight of ash in dried yeast, employing for this purpose the result obtained in the previous experiment.

Test portions of the ash qualitatively for the presence of phosphoric acid, potash and magnesia, of which it is principally composed.

3. *Determination of Nitrogen in Yeast* (Kjeldahl's method, see p. 55).—Weigh very accurately about 0·25 grm. of pressed yeast in a suitable Jena beaker or flask. Heat with sulphuric acid and potassium sulphate, and proceed in the usual manner of a Kjeldahl determination. From the amount of nitrogen obtained calculate the percentage weight present in the yeast both in the pressed state and also when dried. Note that nitrogen is an important constituent of yeast, which must,

therefore, require a considerable amount of nitrogenous food during its growth.

The Nature of the Food Requirements of the Yeast Cell.—Before commencing this study it is necessary for the student to learn the use of the hæmacytometer for the purpose of counting yeast cells.

1. *Use of the Hæmacytometer.*—The form of hæmacytometer recommended is the one known as Thoma's (Fig. 20).

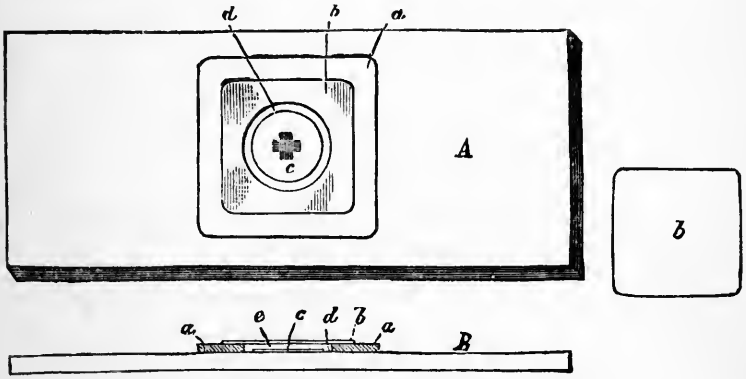


Fig. 20.—Figure of Thoma's Hæmacytometer.

A is a thick glass microscope slide on which a square of glass (*a*) 0·2 mm. thick with a circular hole in the middle is cemented. A circular glass (*c*) 0·1 mm. thick is cemented centrally in this hole, leaving an annular space (*d*) between (*a*) and (*c*). In the middle of (*c*) two sets of equi-distant parallel lines are etched, cutting each other at right angles. There is thus formed a large square with a side of 1 mm. subdivided into 400 small squares each

having a side of 0·05 mm. If a drop of liquid is placed on the square and enclosed by the cover glass (*b*), the depth of the layer of liquid thus formed is 0·1 mm. As the large square has an area of 1 square mm. and the column of liquid above it is 0·1 mm. in depth, the volume of the liquid prism above the large square is thus 0·1 cubic mm. ; and as the large square is subdivided into 400 small squares, the prism of liquid above each small square is $\frac{1}{4000}$, or ·00025, of a cubic mm.

If now a liquid placed in the cell contains yeast cells, these after standing a short time will settle on the surface of (*c*), and the cells in the prism of liquid over the large etched square will settle on the square. If the cells resting on the large square are then counted under the microscope the number found will represent the number contained in 0·1 cubic mm. of the original liquid. The same remarks apply also to the small squares, each of which represents $\frac{1}{4000}$ of a cubic mm. of liquid.

The above description indicates very briefly the nature of Thoma's hæmacytometer and the manner in which it is used ; for fuller information the student is referred to Klöcker's *Fermentation Organisms*, p. 126. But it should be understood that when the student is learning the use of the hæmacytometer and other special operations connected with fermentation work referred to later on, he must work under the direction of a competent

instructor, for personal guidance in the details of such work is necessary for all but the most experienced students.

Experiment. — Weigh out 1 grm. of pressed yeast in a small beaker, and mix it with a little water to a thin liquid. Transfer the liquid yeast and the washings of the beaker to a 100 c.c. flask, and dilute with water to a volume of 100 c.c.

Shake the flask violently to disseminate the yeast cells evenly throughout the liquid. If this cannot be done sufficiently well owing to the limited capacity of the 100 c.c. flask, transfer the liquid to a dry flask of larger capacity. Immediately after shaking the flask, transfer 50 c.c. of the liquid by means of a pipette into another flask and dilute with 50 c.c. of water. Again shake violently and remove as rapidly as possible a small drop with a capillary glass tube or pointed glass rod to the hæmacytometer, and cover it at once with the glass (*b*). Note that the drop taken must be of sufficient size to touch the lower surface of the glass (*b*), but must not be so large as to run into the annular space (*d*). If the size of the drop does not conform to these conditions, it must be wiped off, and the experiment repeated. When a satisfactory drop is obtained, transfer the hæmacytometer to the stage of a microscope placed in an upright position, and allow a few minutes for the yeast cells to settle. Then proceed to count the cells under a $\frac{1}{4}$-inch objective. It will usually be

found convenient to count the number of cells in a perpendicular column of twenty of the smaller squares; but before commencing to do so it is necessary of course to decide with regard to those cells which may happen to touch the lines bounding the column of squares. If all those touching the lines bounding the left hand and top of the column are counted, those touching the right hand and bottom lines must be omitted; either this or a reverse method must be adopted, or a correct number will not be obtained. The cells in at least five columns of the smaller squares, (100 squares), must be counted in order to obtain a fair average. After making one count of the cells, the hæmacytometer should be cleaned and the cells counted in a fresh drop. If the numbers obtained agree closely, the determinations may be regarded as satisfactory; if not, further countings of other preparations must be made until close agreement is obtained.

The average number of cells obtained for a small square represents the number present in $\frac{1}{4000}$ or ·00025 of a cubic mm. of the liquid. Calculate the number of cells present in the original 1 grm. of pressed yeast taken, bearing in mind that the original preparation was diluted to one-half its volume before counting.

In order to assist in realising the vast number of cells obtained, calculate the distance a single row of the cells would cover if placed in a straight

line with the cells just touching each other, assuming that the average diameter of a cell is 6μ. A μ, or micro millimeter, equals $\frac{1}{1000}$ or ·001 of a millimeter, and is the unit employed in microscopic measurement.

2. *The Nature of the Food Requirements of the Yeast Cell.*—Three flasks, of similar shape, with a capacity of about 250 c.c. are required for this study.

Introduce into flask (*a*) 100 c.c. of a 10 per cent. solution of pure cane-sugar in distilled water.

Introduce into flask (*b*) 100 c.c. of a 10 per cent. solution of cane-sugar in Pasteur's nutritive mineral solution. This solution is prepared by dissolving the following constituents in 1000 c.c. of water :—

K_2HPO_4 - - - - -	2·0 grms.
$MgSO_4$ - - - - -	0·2 ,,
Ammonium tartrate - -	10·0 ,,
Calcium phosphate - - -	0·2 ,,

Introduce into flask (*c*) 100 c.c. of a 10 per cent. solution of cane-sugar in yeast water.

Weigh out 5 grms. of pressed yeast, and after mixing it thoroughly with a little water, dilute to 100 c.c. with more water, and agitate the mixture violently. Now add 5 c.c. of the diluted yeast to each of the flasks (*a*), (*b*) and (*c*), so that the contained solutions are yeasted with an equal number of cells in an equal volume of liquid. Close the flasks with cotton-wool stoppers, and place them in an incubator at a temperature of about 20°. At

the same time add 5 c.c. of the diluted yeast used in the above experiments to 100 c.c. of water, and after agitation count the cells in the hæmacytometer. Note that the number thus obtained for the standard volume of $\frac{1}{4000}$ cubic mm. is the number of cells present in the same volume of the different solutions in the three flasks at the commencement of the experiment. Allow the three flasks to remain in the incubator for about forty-eight hours to admit of cell multiplication being complete, and then count the yeast cells present in each solution in the usual manner. The solutions (*b*) and (*c*) may require dilution before counting (see Klöcker, p. 127). Compare the new numbers found for the standard volume of $\frac{1}{4000}$ cubic mm. in each experiment with those originally obtained, and note that yeast supplied with cane-sugar as its only food supply exhibits little or no signs of multiplication, but that yeast supplied with cane-sugar and Pasteur's mineral solution, or cane-sugar and yeast water multiplies freely.

The Maximum Number to which Yeast Cells Multiply in a Nutritive Solution is not Directly Dependent on the Number of Cells Originally Introduced. —Two flasks (*a*) and (*b*), of 250 c.c. capacity, each containing 100 c.c. of a boiled and filtered malt wort of a specific gravity of about 1055°, are prepared; a mixture of 5 grms. of pressed yeast in 100 c.c. water is also prepared as in the previous experiment. Add 5 c.c. of the diluted yeast and 5 c.c. of water to flask (*a*), and 10 c.c. of the diluted yeast to flask (*b*). It will be noticed that flask (*b*) then contains twice as many yeast cells in a given volume

as are present in flask (*a*). Place the flasks in an incubator heated to about 20°, after shaking them to mix the yeast. As a check experiment add 10 c.c. of the diluted yeast to 100 c.c. of water, and count the number of cells present.

When yeast multiplication is complete (usually in forty-eight hours), proceed to count the yeast cells present in (*a*) and (*b*), noting that the number of cells present will probably necessitate dilution of the wort with water to four or five times its volume previous to counting. After correcting for dilution, compare the number of cells found per standard volume in both flasks, and note that the numbers found are very similar, although the number of cells with which (*a*) was yeasted was only half that of (*b*).

From the counting experiment made as a check at the commencement of the study, the number of yeast cells per standard volume present in (*a*) and (*b*) when the experiments were started may be ascertained. Compare the numbers with the numbers found for (*a*) and (*b*) at the close of the experiment, and note that the multiplication of each original cell in (*a*) has been about twice as great as in (*b*).

The Maximum Number to which Yeast Cells Multiply in a Nutritive Solution is not Directly Dependent on the Amount of Yeast Food Present.—Prepare two flasks (*a*) and (*b*) of 250 c.c. capacity, (*a*) containing 100 c.c. of a malt wort of specific gravity 1075°, and (*b*) 100 c.c. of the same malt wort diluted with water to a specific gravity of 1050°. Add to (*a*) and (*b*) 10 c.c. of a mixture of yeast and water, prepared as in the previous experiment, and, after shaking the flasks well, place them in an incubator heated to about 20°. When yeast growth is complete, remove the flasks from the incubator and count the number of cells present in the usual manner. Observe that although the amount of fermentable matter and yeast food present in (*a*) exceeds the amount in (*b*) to the extent of 50 per cent., the numbers of yeast cells found in (*a*) and (*b*) are very similar.

The student's attention is specially called to the technical bearing of the last two studies.

Removal of Nitrogen from Malt Wort during Fermentation.—Prepare a boiled, hopped malt wort of specific gravity 1055°. Measure 100 c.c. of the wort into a 250 c.c. flask, and add about 0·25 grms. pressed yeast. Close the flask with a cotton-wool plug, and place it in an incubator at 20° to ferment.

Evaporate 10 c.c. of the same wort and proceed to determine the amount of nitrogen it contains, using Kjeldahl's method.

As soon as the fermentation in the flask is complete, filter some of the fermented wort until it is quite bright. Evaporate 10 c.c. and determine its nitrogen contents.

Compare the amount of nitrogen found in 100 c.c. of the wort before and after fermentation, and note that the amount which has disappeared has been assimilated during yeast growth. Compare this result with the determination of nitrogen in yeast made previously (p. 132).

Influence of Temperature on the Development and Fermentative Power of Yeast.—Prepare three flasks (*a*), (*b*) and (*c*) of 250 c.c. capacity, each containing 100 c.c. of malt wort of a specific gravity of about 1055°, and add to each flask 5 c.c. of a mixture of yeast and water containing 5 grms. pressed yeast in 100 c.c.

Put (*a*) in a cool cellar at a temperature of about 12° (54° F.), and (*b*) in an incubator at a temperature of about 30° (86° F.). Raise the temperature of (*c*) to 60° (140° F.) in a water-bath for

fifteen minutes, and afterwards place it in the incubator with (*b*). At the same time add 5 c.c. of the diluted yeast to 100 c.c. of water, and count the number of cells present in the standard volume of $\frac{1}{4000}$ cubic mm.

In eighteen to twenty-four hours add 0·1 grm. of salicylic acid to each of the flasks to arrest fermentation, and, after shaking violently, count the number of cells present in $\frac{1}{4000}$ cubic mm. of each solution. Filter about 70 c.c. of the wort in each flask and determine its specific gravity or "attenuation".

Compare the number of cells found with the number originally introduced, and note that the increase in (*b*) is much greater than in (*a*), thus indicating that a temperature of 30° is much more favourable to yeast multiplication than a temperature of 12°. Observe also that the "attenuation" of (*b*) is much lower than (*a*), showing that fermentation has proceeded more rapidly at the higher temperature.

On the other hand, the number of yeast cells in (*c*), and the "attenuation" of the wort have remained unaltered from the commencement of the experiment, indicating that the short period of heating to 60° has destroyed the multiplying power and the fermentative power of the yeast originally introduced. Note that this experiment illustrates the principle of the technical process known as "Pasteurisation".

Actions of Some of the Enzymes Present in the Yeast Cell.

1. The power possessed by the yeast cell of breaking down or fermenting sugar into alcohol and carbon dioxide has been shown to be effected by an enzyme, zymase, but the experimental difficulties associated with a demonstration of this prevent it at present from becoming a student's laboratory experiment.

2. *Invertase.*—The student has already become acquainted with the actions of this enzyme when studying the inversion of cane-sugar (see p. 83).

3. *Maltase.*—The presence of this enzyme in the yeast cell, unlike invertase, cannot be demonstrated without special preparation of the yeast.

Spread a very thin layer of about 20 grms. of finely crumbled, well-pressed yeast on a porous plate, and dry it in a vacuum dessicator over sulphuric acid for two or three days. Powder the dry mass very finely in a mortar and transfer to an air-bath, the temperature of which must be raised very slowly (in about two hours) to 50°, at which point it must be kept for one hour.

To demonstrate the presence of maltase in the prepared yeast, add about 0·5 grm. of the powder to 100 c.c. of a solution of about 5 per cent. of maltose of known rotatory power containing 0·5 c.c. of toluene as an antiseptic (chloroform must not be used as it prevents the action of maltase). Cork the flask containing the solution and keep it at a

temperature of 35°. In three or four hours filter and examine the filtered solution in the polarimeter. A considerable fall in the rotation of the solution will be observed, indicating the conversion of maltose into dextrose. From the fall in rotation calculate the amount of maltose converted into dextrose. Confirm the presence of dextrose by preparing its osazone (see p. 81).

Auto-Digestion of Yeast.—Crumble about 1 lb. of freshly pressed yeast as finely as possible, and after heaping it together on a sheet of filter-paper, introduce the bulb of a chemical thermometer into the centre of the mass. Observe the temperature of the mass from time to time, and note that it tends to increase owing to the respiration of oxygen by the yeast cells and the combustion of reserve material.

Pack into a broad-mouthed 10 or 20 oz. bottle some freshly pressed brewer's yeast, as firmly as possible, until the bottle is about three-parts filled. Close the bottle by tying a piece of filter-paper over its mouth, and put it aside in some place where it will be kept at a temperature of 20° to 25°. Observe from day to day that the mass of yeast gradually liquefies until in the course of several weeks it consists of a dark muddy liquid. Note that the change is not due to putrefaction, for the liquid has no unpleasant smell and few or no living organisms are to be found in it. The change may be regarded as an auto-digestion of the yeast by its

own proteolytic enzymes. If the liquefied yeast is kept for some months small concretions of tyrosine will usually be found in the deposit which have resulted from the breaking down of the proteids of the yeast.

The liquid expressed from the auto-digested yeast may be utilised as a source of invertase if required.[1]

PART II.

The Following Course of Experiments Constitutes a Study of the Morphology and Life History of Some of the More Important Micro-Organisms of Fermentation, and Introduces the Student to the Special Methods Employed in the Study of Fermentation Organisms.

In the following studies description of detail has been avoided as far as possible, for the student can obtain much of the information he will require from the books to which references are given in the text. But books alone are an insufficient guide for a beginner, and it is essential that he should carry on his studies under the supervision of an instructor who is able to assist him in many details of his work which require personal demonstration.

It is assumed that the student is able to work

[1] See C. O'Sullivan and Tompson, *Journ. Chem. Soc.*, 1890, lvii., p. 869.

in a laboratory properly equipped for the special character of his studies, and that he already possesses some knowledge of the principles and methods of bacteriology.

Preparation of the Culture Media Commonly Employed in the Study of Fermentation Organisms.—It is desirable for the student to prepare stocks of culture media when commencing the following studies, in order to acquire a practical knowledge of the methods of preparation and sterilisation usually adopted. Subsequently it will save much time if he is supplied with the media ready prepared.

Hopped Malt Wort (see Klöcker, p. 71).—Boiled hopped wort from a brewer's copper may be used with advantage. The wort should be diluted with water to a specific gravity of about 1050. The wort must be filtered until it is quite bright, and should remain free from deposit after sterilisation. The wort may be stored in flasks, closed with cotton-wool plugs.

Prepare 2 liters of wort and store in four flasks after sterilising in the usual manner.

Yeast Water.—Heat a liter of tap water in a 2 liter flask over a gas flame, and when it is boiling briskly add gradually about 70 grms. of pressed yeast. Boil the mixture for fifteen minutes, and afterwards allow it to digest on a boiling water bath for one hour. Filter until quite bright and store in the same manner as malt wort.

Meat Extract.—Mince finely 500 grms. of lean meat, and mix it with a liter of water in a beaker. Keep the beaker over-night in an ice cupboard or cold cellar. Strain the liquid from the meat through a cloth, and boil the filtrate to remove the albuminous bodies present. Add 5 grms. of sodium chloride and 10 grms. of peptone to the liquid, and filter. Render the solution *very feebly* alkaline with sodium carbonate, and make up its volume to 1000 c.c. with water. Divide into two equal volumes, and store in two flasks after sterilisation in the usual manner.

For other Liquid Media sometimes used see Klöcker, pp. 79, 80.

Wort Gelatin.—Soak 100 grms. of best French gelatin in cold water in a large beaker for an hour. Pour away the water, and replace with a liter of the malt wort already prepared. Transfer the beaker to a boiling water bath, and heat until the gelatin has completely dissolved in the wort. Cool the wort to about 50°, and add to it half the white of an egg, previously diluted and well mixed with a little of the wort. After mixing the solutions thoroughly, raise the mixture to the boiling point and boil for two or three minutes. Filter the solution through flannel, and the wort gelatin will be sufficiently bright for many purposes. For some purposes, however, perfectly clear wort gelatin is required. To prepare it, filter about 500 c.c. of the wort gelatin when hot through a paper-filter

placed in a hot-water funnel. Store the wort gelatin in flasks, and sterilise in the ordinary steam steriliser; note that an autoclave must not be used for this purpose, as the high temperature to which the wort gelatin is exposed injures its setting power (see Klöcker, pp. 81-85).

Preparation of Freudenreich Flasks and Test-Tubes Containing Malt Wort and Gelatin Malt Wort.—Sterilise in the usual manner a number of

Fig. 21.—Freudenreich Flask.

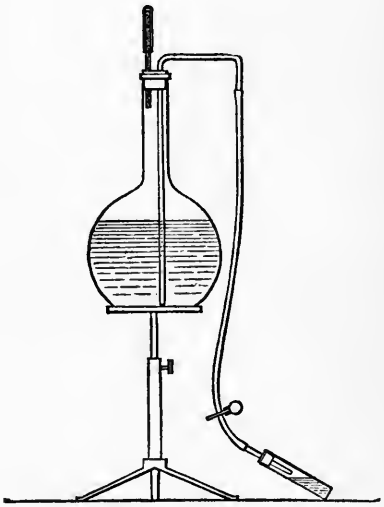


Fig. 22.—Holder for Sterile Water.

Freudenreich flasks and test-tubes, plugged with cotton wool, in the hot air steriliser at 150° (302° F.). When cool measure into the flasks from a burette or pipette, 5 c.c. of malt wort, or malt wort gelatin rendered liquid by heat. Measure into the test-tubes 10 c.c. of the same media. When filling the flasks and test-tubes avoid wetting the necks

of the vessels with the culture media. Sterilise with steam in the usual manner, noting that wort gelatin should be heated for only sufficient time to ensure perfect sterilisation, as prolonged heating affects its setting property.

Sterile Water.—A supply of sterile water is frequently required during work with micro-organisms. A holder containing a supply of sterile water should be prepared. For description see Klöcker, p. 67.

A. THE SACCHAROMYCETES AND LOWER FUNGI.

Study of the Morphology of a Yeast Cell.—A sample of ordinary matured brewer's yeast is required. Place a small drop of water on a microscope slide and add to it a minute drop of yeast, so that the appearance of the water drop is slightly milky. Cover with a clean cover-glass and examine under the microscope with a $\frac{1}{8}$-inch objective.

Observe that the yeast cells are spherical or slightly elliptical in shape. Like most living cells, they consist of protoplasm, or living matter, enclosed by a membrane—the cell wall.

To render the cell wall easily visible, remove the slide from the microscope and press the centre of the cover-glass gently, in order to burst some of the yeast cells. Examine again under the microscope and the walls of the burst cells will now be visible as very thin transparent membranes.

Examine another preparation of yeast in water,

and note that the contents of the cells are not homogeneous, but that one or two round, clear spaces of considerable size are visible in each cell which have the appearance of being of less density than the protoplasm. These spaces are called *vacuoles*, and are filled with cell sap.

Examine the protoplasm carefully and it will be noticed that a number of dense particles lie embedded in it, giving it a mottled appearance. These particles, or granules, are composed of fatty matter enclosed in a layer of albuminous material. The granules situated in the protoplasm are without motion, but granules may be frequently observed within the vacuoles which possess a Brownian movement indicating the fluid condition of the cell sap in which they are floating. (See *Technical Mycology*, Lafar, vol. ii., p. 145, concerning the "Anatomy of the Yeast Cell".)

The protoplasm of the yeast cell, like that of all living cells, is composed of a central body—the nucleus—surrounded by cytoplasm; but the nucleus of the yeast cell is only rendered visible by certain staining processes which are somewhat difficult of execution (Klöcker, p. 89).

Dry, stain and mount a preparation of the yeast in the usual bacteriological manner (Klöcker, p. 88).

Having now studied the general anatomy of the mature yeast cell, the student should proceed to

observe the changes which take place in the appearance of the cell during the various stages of its growth.

Add a little of the mature yeast already examined to some hopped malt wort in a small flask, and place the flask in an incubator kept at a temperature of about 25°. Proceed to examine the yeast cells from time to time under the microscope, at first at intervals of one hour, and afterwards at longer intervals. Note the changes which rapidly take place in the general appearance of the cells. The granular appearance of the protoplasm disappears and the cells become very transparent; the walls of the cells also appear to become thinner.

Note also the changes in appearance of the vacuoles. Observe that the first signs of reproduction of the yeast cell by budding is evidenced after two or three hours by a slight protrusion on one side of the cell. The bud gradually enlarges until a full-sized cell is formed from the mother cell; but this method of reproduction will be followed better when employing the drop culture method described below. Observe as fermentation draws to a close that the protoplasm of the yeast cells becomes more granulated, and the cells return to the original appearance of the mature yeast with which the present study was commenced.

Determine the average size of the yeast cells by means of the micrometer eye-piece of the micro-

scope, and express in micromillimeters (1 μ or micromillimeter equals ·001 mm.)

Growth of Yeast in a "Drop Culture".—*Requirements* for the preparation of a "drop culture".

Thin glass rods in a tin box with a glass cover.
A glass plate and glass bell jar or beaker to act as a cover.
Microscope slides and Böttcher's moist chambers (see Klöcker, p. 69), or hollow-ground slides.
Microscope cover-glasses in small Petri dish (for cleaning cover-glasses, see Klöcker, p. 87).
Freudenreich flasks of sterilised wort.
Vaseline.
Forceps.

Fig. 23.—Moist Chamber.

Sterilise the box containing glass rods, and the Petri dish containing cover-glasses, in the hot air steriliser. Sterilise the glass plate and cover with a Bunsen flame, and afterwards sterilise the microscope slides and moist chambers in the same manner, holding them in the flame with the forceps. Place the slides and moist chambers after sterilisation on the glass plate underneath the cover.

Preparation of a Drop Culture.—In special work the preparation of a drop culture is conducted in a sterile chamber (see Klöcker, p. 21), but this precaution is not necessary in the ordinary course of a student's work, and therefore reference to this part of the process is omitted here.

Take a glass slide from below the cover with the forceps, and lay it on the top of a Petri dish which has been sterilised by being passed through a Bunsen flame. Place three drops of sterile wort from a Freudenreich flask side by side on the glass slide by means of a sterile glass rod. A trace of the yeast to be examined is now added by means of the glass rod to one of the drops of water and the whole well mixed. A little of the first drop is then added to the second by means of a second sterile rod. After mixing, a little of the second drop is added to the third by means of another sterile rod. A small drop of the final mixture is now placed in the centre of a cover-glass, and the glass is then rapidly inverted and fixed on the ring of a moist chamber, the edge of which has been previously smeared with vaseline. A small drop of wort from the Freudenreich flask should be placed on the bottom of the moist chamber previous to the fixing of the cover-glass, in order to keep the air of the chamber moist.

Examine the drop culture under the microscope, which must be in an upright position. The yeast cells present should be so few in number that not more than one or two are visible in each field of view. If the cells are too numerous, another drop culture must be made with further dilution of the culture.

Study the cells from time to time as they multiply by budding, and make drawings. The

culture when not under examination should be kept in an incubator at 20°-25° (68°-77° F.). If it is desired to follow the multiplication of one particular cell it must be carefully marked, or the slide must be fixed on the microscope, and the latter placed in an incubator or other suitably warm place. (For mode of propagation of yeast by budding, see Klöcker, p. 191.)

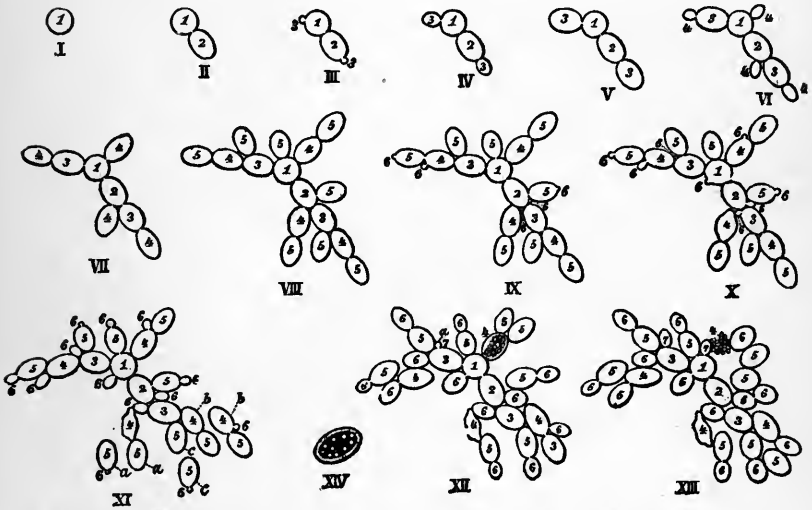


FIG. 24.—Multiplication of top yeast: I., $\frac{28}{6}$, 7 P.M.; II., $\frac{27}{6}$, 8 A.M.; III., 9 A.M.; IV., $10\frac{1}{3}$ A.M.; V., 12 noon; VI., $3\frac{1}{2}$ P.M.; VII., 8 P.M.; VIII., $\frac{28}{6}$, 8 A.M.; IX., 10 A.M.; X., 11 A.M.; XI., 1 P.M.; XII., $\frac{29}{6}$, 8 P.M.; XIII., $\frac{30}{6}$; XIV., $\frac{2}{6}$, 12 o'clock. (After Mitscherlich.)

A Study of Some of the Well-recognised Races of Yeasts and Torula.—When the student is familiar with the appearance and ordinary mode of reproduction of brewer's yeast, he should then proceed to study some of the well-recognised races and species of yeasts and torula. Inoculations

from pure cultures of these should be obtainable in the laboratory in which the student works, and should at first be grown in tubes of malt wort. The general appearance of the organisms should be studied under the microscope and also their modes of development in drop culture, careful drawings being made at all times. A selection from the following list of organisms is recommended :—

Saccharomyces cerevisiæ. Top fermentation race.
,, ,, Saaz and Logos; low fermentation races. (See Klöcker, p. 220.)
,, Pastorianus I., II., and III. (See Klöcker, pp. 254-256.)
,, ellipsoideus I. and II. (See Klöcker, pp. 257-259.)
,, Marxianus. (See Klöcker, p. 261.)
,, anomalus. (See Klöcker, p. 262.)
Schizo-saccharomyces Pombe. (See Klöcker, p. 269.)
,, octosporus. (See Klöcker, p. 270.)
Saccharomyces (?) apiculatus. (See Klöcker, p. 294.)
Mycoderma vini. (See Klöcker, p. 297.)
Torula. (See Klöcker, p. 289.)

A Study of Some of the more Common Species of Moulds.—The close relationship of the saccharomycetes to the moulds, and the important fermentation changes induced by many species of moulds, renders it most desirable for the student to study some of the more important types of these organisms.

Inoculations from pure cultures of the moulds should be made in malt wort and on wort gelatin, and the modes of growth of the organisms studied.

At the same time drop cultures should be made with spores or mycelia of the moulds, and the different stages in the growth and development of the organisms carefully followed under the microscope. Drawings should be made throughout these studies.

A selection from the following moulds is recommended :—

Mucor racemosus. (See Klöcker, p. 179.)
Mucor (Amylomyces) Rouxii. (See Klöcker, p. 181.)
Penicillium glaucum. (See Klöcker, p. 278.)
Aspergillus glaucus. (See Klöcker, p. 274.)
Monilia candida. (See Klöcker, p. 298.)
Oidium lactis. (See Klöcker, p. 303.)
Dematium pullulans. (See Klöcker, p. 305.)

Preparation of Spore Cultures of the Saccharomycetes.

Requirements :—

A pure culture of yeast twenty-four hours old.
Sterilised gypsum blocks in covered glass dishes. (See Klöcker, p. 64.)
Sterilised water.
Sterilised pipettes. (These may be made by drawing out thin glass tubing in a gas flame.)

In order to induce sporulation in yeast, the culture employed must be young and vigorous (see Klöcker, p. 122).

Inoculate a small flask or test-tube of sterile wort from a pure culture of top fermentation yeast, and keep it in an incubator at 25° (77° F.) for twenty-four hours, in which time a sufficient deposit

of freshly formed yeast for the purpose of the experiment will probably be found.

Sterilise the gypsum blocks and dishes in the hot-air steriliser for an hour at 110° to 115° (230° to

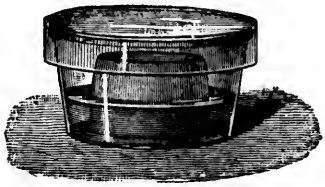


Fig. 25.—Gypsum Block.

239° F.). The temperature must not rise above 120° (248° F.) or the blocks may be spoiled by dehydration of the gypsum.

The small pipettes should be wrapped separately in filter-paper and sterilised at about 150° (302° F.).

To prepare the spore culture, pour away the supernatant fermenting liquid from the yeast culture, and take out a small quantity of the sedimentary yeast with a pipette and spread it in a thin layer on a gypsum block. Pour water from the sterilised water-holder into the dish in which the gypsum block is placed until it is about two-thirds filled. During these manipulations uncover the dish for as short a time as possible. Place the covered dish in an incubator at 25° (77° F.). Examine the culture after twenty-four hours under the microscope for the formation of spores, removing for this purpose a little of the yeast with a sterilised needle and mounting it in water on a glass slide (see

Klöcker, p. 122). If spores are not found, examine at intervals of six or eight hours, and note the time when spore formation is first observed.

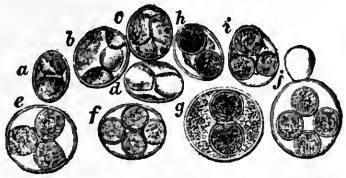


FIG. 26.—*Saccharomyces cerevisiæ I.*, Hansen. First stages of development of the spores. $\frac{1000}{1}$. (After Hansen.)

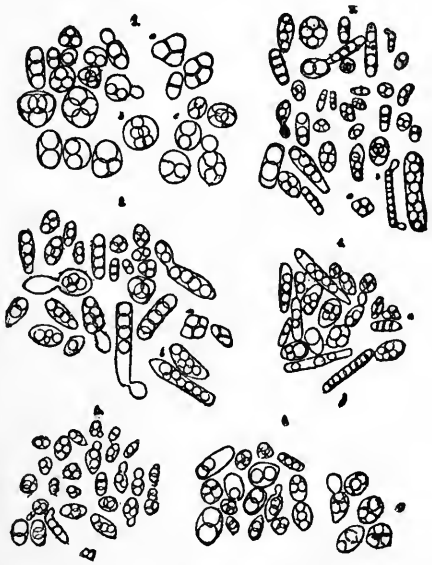


FIG. 27.—Saccharomycetes which form ascospores. 1. *Sacch. cerevisiæ I.* 2. *Sacch. Pastorianus I.* 3. *Sacch. Pastorianus II.* 4. *Sacch. Pastorianus III.* 5. *Sacch. ellipsoideus I.* 6. *Sacch. ellipsoideus II.* *a*, Cells with septa; *b*, cells with more than normal number of spores; *c*, cells distinctly beginning to sporulate. About $\frac{500}{1}$. (After Hansen.)

Make spore cultures of the various species of yeasts which are being studied, and note the different times at which spore formation is first observed (see Klöcker on spore formation, pp. 202 *et seq.*).

Observe the character of the spores in the different species.

Prepare a drop culture of the spores of a yeast in malt wort, and observe the germination of the spores (see Klöcker, pp. 205 *et seq.*).

To make permanent stained preparations of sporulating yeast, distribute the spores mixed in a small drop of water on a clean cover-glass, and dry and fix in the usual manner (see Klöcker, p. 88). Place the cover-glass in a watch-glass containing carbol fuchsine solution (see Klöcker, p. 92) and boil gently for a few minutes. Wash in water, and afterwards for a very short time in dilute hydrochloric acid. Rinse in water, dry, and mount in Canada balsam. The spores of the yeast cells should then appear red under the microscope, and the remaining parts colourless. Double staining can be attempted, if desired.

Film Formation of the Saccharomycetes.—Inoculate pure cultures of the different species of yeast which are being studied into small flasks half-filled with sterilised wort and lightly stoppered with cotton wool. Allow the cultures to stand at room temperature in a place where they will not be disturbed in any way. It is essential for the formation of film growths that air should have free access to the cultures. The time of development of the film formation of a yeast varies with the species, and with the temperature at which the culture is kept (see Klöcker, p. 125 and p. 194). Note the characteristics of the film growths of the different species of yeast, and the manner in which the forms of the cells usually vary from those of the original submerged cultures.

Preparation of Pure Cultures of Yeasts and Moulds.

Gelatin Plate Method of Culture.

Requisites :—

Tubes of sterile wort gelatin.
Sterilised Petri dishes.
Freudenreich flasks containing sterile water.
Inoculating needles.
Sterilised pipettes and glass rods.

Transfer with a sterilised needle a small inoculation of brewer's yeast to a Freudenreich flask containing sterilised water, and shake well in order to distribute the yeast cells evenly throughout the volume of the liquid. Melt the wort gelatin in three test-tubes by placing them in water at about 30° (86° F.), and burn the surface of the cotton-wool plugs of the tubes. Remove the plug from the first tube and add with a pipette 10 drops of the diluted yeast. Mix thoroughly, and with a sterilised rod or wire transfer one drop of the mixture to the second tube. Mix, and transfer one drop of the mixture from the second tube to the third tube. When agitating the tubes to distribute the yeast, take care not to cause the formation of foam in the liquid wort gelatin. Pour the contents of the three tubes into three Petri dishes, keeping them uncovered for as short a time as possible. Mark the dishes with the dilution used, and place them on a level surface until the gelatin is set. Keep the cultures in an incubator at a temperature of about 20° (68° F.) until the yeast

colonies develop. Note the relative size and number of the colonies obtained in the three cultures.

To make a wort culture from any one of the yeast colonies selected, sterilise a small piece of platinum or brass wire by holding it in a gas flame in forceps, and when cool dip it into the selected colony so that some of the yeast adheres to the wire. Transfer the piece of wire to a flask or tube containing sterile wort, and allow the culture to grow at a suitable temperature (see Klöcker, pp. 105 *et seq.*).

The gelatin plate method of culture is specially suitable for the preliminary separation of mixed cultures of yeast, but there is no guarantee when employing the method that each colony found is a pure growth, owing to the possibility of two or more cells of different species of yeast being close together when the original gelatin culture was made. In order to obviate this difficulty and perfect the process, Hansen introduced his single cell method.

Hansen's Method of Pure Yeast Culture from a Single Cell.

Requisites :—

A sterilised glass plate and bell jar or other cover.
Sterilised glass rods.
Sterilised Böttcher's chambers.
Sterilised cover-glasses divided into numbered squares, or plain for use with cell-marker.
Freudenreich flasks of sterilised wort gelatin, and of sterilised water.

Mix a drop of fresh yeast with sterilised water in a Freudenreich flask, shake well and dilute still further by transferring a drop of the mixture to a second flask of water. Again mix by shaking, and, if the liquid then appears slightly opalescent, the right dilution has probably been obtained. Transfer a drop of the mixture to a Freudenreich flask containing liquefied wort gelatin, and mix thoroughly. Then spread a drop of the wort gelatin mixture

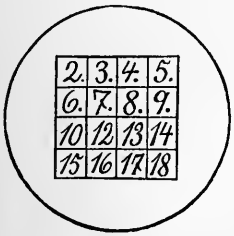


FIG. 28.—Squared Cover-Glass.

FIG. 29.—Klönne and Müller's Object Marker.

in a thin layer on a cover-glass by means of a glass rod, and place the glass on the glass plate underneath the bell jar and leave until the gelatin has set. Prepare a Böttcher chamber by placing a small drop of water at the bottom of the cell and smearing the edge of the ring with vaseline. Then reverse the glass with the gelatin film and adjust it to the ring of the chamber. The preparation should then be transferred to the microscope for examination. The lowest power objective with which the yeast cells can be distinctly seen should

be employed. Those cells are chosen for the purpose of obtaining colonies which are several millimeters apart from other cells, and their position must be carefully recorded. If a cover-glass divided into squares is employed, a diagram should be made indicating the position of the cells chosen; if a plain cover-glass is used, the cells must be marked with the cell-marker (see Klöcker, p. 33).

After marking the position of several cells, keep the culture at a temperature of about 20° (68° F.), and examine it from day to day with the microscope as the cells multiply, in order to be sure that no cells in the immediate vicinity of the colonies have been overlooked. When the colonies are large enough, a pure culture in wort may be obtained from each colony by inoculation in the manner described for gelatin plate cultures (see above).

Modifications of Hansen's Method of Pure Yeast Culture from a Single Cell known as "Lindner's Droplet Culture," and "Schönfeld's Method" are sometimes employed.—For description of these processes see Klöcker, pp. 110, 111.

Analysis of Mixed Cultures of Yeast.—It is desirable when the student has become familiar with the ordinary biological methods employed in the study of the saccharomycetes, that he should attempt to separate and identify the forms in a mixed sample. For this purpose he should be supplied with a mixture of two or more well defined forms of yeast by his instructor, who alone

knows what forms are in the mixture. Two methods of analysis may be employed—the gelatin plate method, and the "fractional culture" method. The former is the easier, and is recommended to the inexperienced student for his first attempt. But it has certain disadvantages, as, for instance, some species of yeast develop very slowly in wort gelatin, and in cases of yeasts which are present only in small quantities in a mixture, they are more apt to be overlooked than when the "fractional culture" method is employed.

Gelatin Plate Method.—Dilute the mixed culture, which should be a freshly grown one, with sterilised water, and proceed to make plate cultures as described above (p. 159). As long a time as possible should be allowed for the development of the yeast colonies, for, as before remarked, some yeasts develop more slowly than others in gelatin.

When the colonies are well developed, examine their general appearance with a pocket lens, and note any characteristic differences which may be evidenced. Examine cells removed from a number of different colonies under the microscope. Inoculate the colonies which exhibit differences into sterilised wort, and observe the manner in which they develop. Study the spore formation of the different yeast cultures obtained. Endeavour to identify the forms separated (see Klöcker, pp. 133 *et seq.*).

Fractional Culture Method (see Klöcker, p. 101).

Requisites :—

Freudenreich flasks containing 5 c.c. sterilised water.
" " " malt wort.
Small stoppered bottle, sterilised.
Hæmacytometer.
Sterilised pipettes.

Two or three drops of the mixed yeast culture are transferred to a Freudenreich flask of sterile water, and well mixed. The diluted culture is then transferred to a small sterilised glass-stoppered bottle and well shaken in order to separate the yeast cells very thoroughly. A small drop is then transferred to the hæmacytometer, and the number of cells in 1 c.c. of the liquid determined (see p. 133). Dilute the liquid with sterilised water, so that 1 c.c. contains about 1,000 cells, and one drop of the liquid (0·05 c.c.) will then contain about fifty cells. Add 0·05 c.c. (or one drop) of this dilution to a Freudenreich flask containing 5 c.c. of sterilised water, and shake the mixture well. This mixture should now contain approximately one yeast cell in two drops of the liquid. Fifty Freudenreich flasks of sterilised wort are then inoculated, each with one drop of the mixture added by means of a sterile pipette, and kept in a moderately warm place for two days where they will not be disturbed in any way. The flasks are then examined, and those containing a single yeast colony are separated from the rest. After further development of the cultures in these flasks, they are examined microscopically and separated into groups according to their characteristics. Further examination is conducted as with the gelatin plate method described above.

When analysing yeasts in order to determine the presence of wild yeasts, which may be present only in very small quantity, it is often necessary, previous to analysis, to cultivate the original yeast in a cane-sugar solution containing tartaric acid (see Klöcker, p. 135).

A Method of Testing the Pure Cultures of Yeasts obtained by Previous Methods of Analysis for Technical Purposes. — Inoculate the pure cultures to be tested into flasks containing about half a liter of sterilised wort, and incubate at 25° (77° F.) for two days. Sterilise a number of glass cylinders of about 1500 c.c. capacity by burning a little alcohol in them, and pour into each cylinder a liter of sterilised hopped wort of known specific gravity (about 1050°). Inoculate the wort in the different cylinders with the cultures of yeast which have accumulated from the fermentations in the flasks. After covering the cylinders with two or three layers of sterilised filter-paper, allow the fermentations to proceed at about 20° (68° F.). As the fermentations proceed, observe the nature of the different yeasts regarding their high or low fermentation characteristics. Note the behaviour of the yeasts towards the close of the fermentation as to whether they separate quickly, or remain floating in the liquid. Note the flavour and smell of the completely fermented worts. Determine the "attenuation" of the fermented worts, and also the amount of alcohol present.

By means of the above experiments information of technical value can be often obtained in the laboratory regarding the properties of pure yeast cultures. (See Jörgensen's *Practical Management of Pure Yeast*, p. 37.)

Experiments on the Varying Powers of Hydrolysis and Fermentation possessed by Different Species of Yeasts.—Experiments should be made by growing suitable species of yeasts in solutions of such carbo-hydrates as cane-sugar, dextrose, maltose and lactose, in yeast water, in order to study their powers of selective fermentation. One hundred c.c. of a 3 per cent. solution of the carbo-hydrate is usually a convenient amount to employ in these experiments. The solutions should be sterilised in small flasks plugged with cotton-wool, and inoculated in the usual manner. Examination of the flasks during the course of the experiments is often sufficient to indicate if fermentation is proceeding, but in some cases, which should suggest themselves to the student, it is desirable to determine the nature of the sugars present in the solutions when fermentation is proceeding. The means he should employ for this purpose will be already familiar to him. During the continuance of the fermentation experiments the flasks should be kept in an incubator at 25° (77° F.).

Yeasts recommended for experiment are:—

Sac. cerevisiæ.

Sac. anomalus (see Klöcker, p. 263).

Schizo-saccharomyces octosporus (see Klöcker, p. 271).

B. THE SCHIZOMYCETES OR BACTERIA. (See Klöcker, p. 271.)

The Morphology of a Bacterium.—Prepare a culture of Bacillus subtilis (the hay bacillus). Infuse some dry hay in water for two or three hours at the temperature of the room. Pour the infusion obtained into a flask, and close it with a plug of sterilised cotton-wool. Boil the infusion gently for five minutes, allow it to cool, and place it in an incubator at 25° to 30° (77° to 86° F.). In

twenty-four hours a growth of B. subtilis should develop on the surface of the infusion. Transfer with a needle a little of the surface growth to a drop of water on a glass slide, and examine it under a cover glass with a high power objective. Observe that the film is composed of long cylindrical rods or cells. These cells, like those of the saccharomycetes, consist of protoplasm enclosed by a cell wall. Also, as with yeast cells, granules and vacuoles occur in the protoplasm, but owing

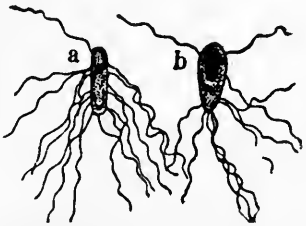


FIG. 30.—*Clostridium butyricum*, Prazmowski. Butyric acid bacterium with flagella. *a*, Vegetative motile cell; *b*, sporulating motile cell. $\frac{1200}{1}$. (After Alfr. Fischer.)

to their small size are only seen with very high magnification. (See Lafar's *Technical Mycology*, vol. i., p. 42; also Klöcker, p. 312.)

Note the transverse partitions or walls subdividing some of the cells. These indicate the ordinary way in which the cell multiplication of bacteria takes place. In the cell of a fully developed bacterium one or more transverse septa form, dividing it into two or more separate cells; these may remain united as a chain of cells, or separate into individual cells. This method of multiplication of bacteria by *fission* differs essentially

from multiplication by budding which characterises the saccharomycetes. Note that the long chains of bacteria in the culture are motionless, but that some of the single cells possess an active swimming motion. The motion is due to the action of *flagella*, fine hair-like processes, which are attached to the cells (see Klöcker, p. 313). The flagella are too fine and transparent to be observed without preparing and staining the cells by certain processes which are somewhat difficult of execution.

Make a Stained Preparation of B. subtilis showing the Flagella.—Dry and fix the organisms in the usual manner on a cover-glass. Prepare a mordant by mixing one volume of extract of logwood (1-8) with two volumes of a 20 per cent. solution of tannin, to which a few drops of a saturated solution of ferrous sulphate have been added. Place some drops of this solution on the cover-glass preparation and warm over a flame until steam is formed. Wash in water and stain with Löffler's gentian violet solution (see Klöcker, p. 92). Wash again in water and mount in the usual manner. The preparation should be examined under a $\frac{1}{12}$ inch immersion lens.

Make a Stained Preparation of B. subtilis.—The preparation should be stained and fixed in the same manner as yeast (see Klöcker, p. 88).

For Gram's method of staining bacteria for the purpose of identification see Klöcker, p. 90.

Spore Formation.—When the culture of B. subtilis is two or three days old, highly refractive spores will be observed in many of the cells. These endospores, similar to the endospores of

the saccharomycetes, are not easily stained by dyes.

Make a cover glass preparation of the spore-bearing bacteria, and stain with fuchsine or methyl violet in the usual manner. Under these conditions it will be observed that the spores remain uncoloured, whilst the surrounding cell matter is stained. To stain the spores see Klöcker, p. 90.

Prepare a Drop Culture of the Spores of B. subtilis in Hay Infusion.—The spores may be obtained from a culture of B. subtilis several days old.

Study the development of the spores into rods (see Klöcker, p. 318).

A Study of Some of the Well-recognised Species of Acid-producing Bacteria.—A selection from the following list of organisms is recommended :—

Bacillus viscosus. (Klöcker, p. 341.)
Bacillus acidi-lactici. (Klöcker, p. 342.)
Bacillus amylobacter. (Klöcker, p. 343.)
Saccharobacillus Pastorianus. (Klöcker, p. 343.)
Bacterium aceti. (Klöcker, p. 336.)
Bacterium Pasteurianum. (Klöcker, p. 337.)
Bacterium xylinum. (Klöcker, p. 340.)
Sarcina and Pediococcus forms. (Klöcker, p. 331.)

Inoculations from pure cultures of these organisms should be supplied to the student. B. viscosus, B. acidi-lactici, B. amylobacter and Saccharobacillus Pastorianus may be grown in solutions of dextrose (3 per cent.) in yeast water to which a little calcium carbonate has been added. B. amylobacter must

be cultivated under anaërobic conditions. The acetic bacteria should be grown preferably in sterilised fermented wort, or in a mixture of two parts of red wine (claret) and one part water, but they will also grow in unfermented malt wort. Sarcinæ and Pediococci may usually be grown with advantage in neutral meat extract containing a little calcium carbonate. The media used should be sterilised in small flasks or test-tubes. After inoculation in the usual manner the cultures should be kept in an incubator at 30° (86° F.).

The general characteristics of the cultures should be observed as the organisms develop, and from time to time they should be examined under the microscope. Permanent stained preparations of the organisms should also be made. Many special points connected with the life history and chemical actions of the different organisms under observation may be studied with advantage if the student has time at his disposal, and he should now be in a position himself to suggest the nature of his work, and carry it on under the supervision of his instructor.

Examination of Beer Sediments.—See Matthews and Lott (*The Microscope in the Brewery*, second edition, p. 55). The sediments from as many samples as possible of freshly racked beer, matured beer and bottled beer, should be examined under the microscope, and also the sediments of unsound beer, and fretting beer, if they can be obtained.

The student should note that a special knowledge of this class of technical work is very important, and that he will derive much assistance from working under the supervision of a skilled instructor.

The "Forcing" Test as Applied to Beer.—For a full description of this process see Matthews and Lott, p. 128. Note that in place of an open "forcing tray" it is preferable to use an incubator.

When studying the "forcing" test it is desirable for purposes of experience to obtain samples of ale which will develop on forcing growths of the acid-producing bacteria which are common to beer. As it is sometimes difficult to obtain such samples, about 0·5 grm. of calcium carbonate, previously sterilised by heat, should be added to some of the samples before forcing. In the presence of calcium carbonate most samples of beer which would otherwise remain "sound" on forcing develop growths of pediococci and lactic bacteria. The action of the calcium carbonate appears to be due to neutralisation of the natural acidity of the beer, which exerts a retarding influence on the development of acid-forming bacteria.

Micro-organisms present on Barley, Malt and Hops.—Agitate strongly a sample of the barley or hops with well-filtered and sterilised water. Pour off the water quickly, and allow it to subside. Examine the sediment under the microscope (see Matthews and Lott, p. 149, and plates 18 and 19).

Wort gelatin plate cultures of the organisms in the sediments may be made in the usual manner, if required.

Biological Examination of the Air for Technical Purposes.—Both qualitative and quantitative methods may be employed, but for most technical purposes qualitative methods suffice.

1. *Qualitative Method of Examining Air in which Roll Cultures are Employed.*—Sterilise two wide-mouthed 20 oz. bottles plugged with cotton-wool. Place the bottles where the air is to be examined, and remove the cotton-wool plugs, keeping them in a sterilised covered vessel. Allow the bottles to remain open for one hour, and then replace the wool plugs. Liquefy a tube of wort gelatin and one of meat extract gelatin. Into one bottle pour the wort gelatin, and into the other the meat gelatin, and replace the cotton-wool plugs. Agitate the liquefied gelatin in order to incorporate with it the dust collected in the bottles, and prepare roll cultures in both bottles by holding them horizontally in a stream of cold water and rotating them until the gelatin sets round the sides of the bottles as a thin film. Place the bottles in a dark cupboard and keep them at room temperature until colonies of the various organisms contained in the dust of the air have developed. Note the appearance of the colonies, and examine them under the microscope. Classify them as moulds, bacteria and yeasts, or torula, noting the number of each. Study the character of the different species found. Note the difference in number and kind of the organisms which have

developed in malt wort gelatin and in meat gelatin; from a technical point of view those developing in malt wort gelatin are of most importance.

2. *Qualitative Method of Examining Air in which Petri Dishes are Employed.*—Prepare in the usual manner two Petri dishes, one containing wort gelatin, the other meat extract gelatin. Place the dishes where it is desired to examine the air, remove the covers and allow the dishes to remain open for fifteen minutes. Replace the covers, and allow the germs which have fallen on the surface of the gelatin to develop at room temperature in a dark cupboard. Examine the colonies which develop, in the manner described in the previous method (see Klöcker, p. 154).

Mould colonies spread more rapidly when the second method is employed than with the first, and are therefore more apt to interfere with the success of the Petri dish method than when the roll culture method is used.[1]

Hansen's Quantitative Method of Examining Air.—(For description of this process, see Klöcker, p. 150.)

Biological Examination of Water for Technical Purposes.—It has been pointed out by Hansen that the ordinary biological examination of water conducted with meat extract gelatin is not suitable for technical purposes connected with the

[1] The too rapid growth of mould colonies may be controlled by touching them with a small drop of a strong solution of perchloride of mercury in alcohol.

fermentation industries, for the nutrient medium employed favours the development of a large number of organisms which are incapable of development in unfermented or fermented wort and so obscures the main point of the examination, which is the recognition of the special organisms which might occasion trouble in technical practice. For this reason it is advisable to use sterilised wort or sterilised beer as nutrient media in the examination of water for technical purposes. (For a description of the method of examination, see Klöcker, p. 145, and also Hansen, *Practical Studies in Fermentation*, chap. iv., p. 110.)

Biological Examination of Water for Hygienic Purposes.—It is desirable that the student should have some experience of this process.

Examination of Ordinary Tap Water.

Requisites :—

Tubes of gelatin meat extract.
Sterilised Petri dishes.
Sterilised 1 c.c. pipettes (graduated in tenths).

Melt three gelatin tubes, and transfer 0·5 c.c. of the water to one gelatin tube, 0·3 c.c. to the second and 0·1 c.c. to the third. Mix the water and gelatin in the tubes by swinging them round in the hand rapidly, but be careful to avoid frothing. Pour the gelatin in the three tubes into separate Petri dishes, and after marking each culture with the dilution of water used, allow them to set on a level surface. Incubate the cultures at about

20° for three or four days, and count the colonies formed, expressing the result as so many organisms found in 1 c.c. of the original water taken. The nature of the colonies found, whether they consist of liquefying organisms, mould growths, etc., should be carefully noted.

Examination of Polluted Water.—If the water to be tested is polluted with sewage or otherwise contaminated, it will be necessary to use much smaller volumes than those mentioned above for the purpose of inoculation. In order to do this the water must be diluted with sterile water or meat extract. A description of the method usually adopted will be found in any text-book of bacteriology.

SECTION IV.

THE HOP.

The hop (*Humulus lupulus*) is a dicœcious plant, that is to say, the male and female organs of the plant are contained in separate flowers which are borne by different plants. The female plant alone is cultivated by the hop grower, as its flowers alone develop into the well-known hop-cones. The male hop is usually found as a self-sown plant growing wild in the vicinity of hop gardens, and its sole function so far as the formation of hop-cones is concerned is to fertilise the flowers of the female plant with pollen from the anthers of its male flowers, and so lead to the development of seed in the hop-cone.[1]

Examine a Branchlet of a Hop Plant Bearing Female Flowers.[2]—In this state the plant is usually described by the hop grower as being in "burr" (Fig. 3).

[1] For a comprehensive account of the hop plant, see *Agricultural Botany*, by J. Percival, p. 322.

[2] Specimens of the hop in flower may be preserved in spirit for the purpose of examination.

It will be noticed that the flowers have the appearance of small brushes proceeding from a cone composed of small pointed bracts, or scales. The brush-like appearance is due to long stigmata

Fig. 31.—Female Inflorescence of the Hop.

proceeding from the ovaries of the flowers which are concealed within the scale-like bracts. Each ovary is surmounted by two stigmata (Fig. 32 *c*), and has at its base a very small scale-like bracteole (Fig. 32 *b*).

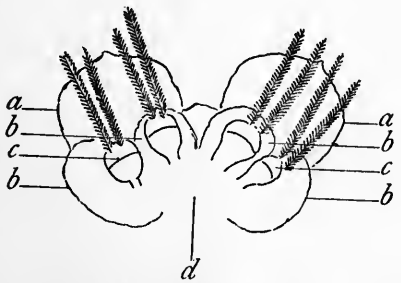


Fig. 32.—Diagram of a Female Flower of the Hop.

After the flowering stage is over, the stigmata shrivel, the main axis of the flower lengthens, and the scale-like bracts (Fig. 32 *a*) and smaller bracte-

oles within these bracts (see Fig. 32 *b*) grow into the leaf-like structures which compose the hop-cone. Owing, however, to the small size and complexity of the hop flower, a practical study of its minute anatomy is too difficult for the ordinary student to attempt, and he is referred to the accompanying diagram (Fig. 32) for information concerning the arrangement of its different parts.

Examine a Hop-Cone or Strobile.—It will be found to consist of a number of leaf-like bracts arranged on a central axis and overlapping one another.[1]

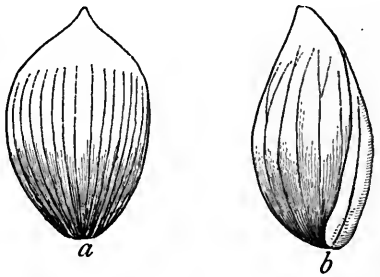


FIG. 33.—Bracts of Hop-Cone. (*a*) Stipular Bract; (*b*) Bracteole.

On examining the bracts closely it will be noticed that they are of two different forms. One form is more symmetrical and rather coarser in

[1] For this study freshly gathered hop-cones, or those which have been dried without pressing should be employed if possible. If such cannot be obtained, some ordinary dried hops should be placed in a closed vessel on a layer of damp sand for forty-eight hours, when they will to a large extent recover their original shape and elasticity. After such treatment fairly perfect hop-cones, in a condition suitable for examination, may usually be obtained.

texture and more thickly veined than the other. This form is called a "stipular bract," and is developed from the pointed scaly bracts of the flower already noticed (Fig. 32 *a*).

The other form of bract is usually rather longer than the stipular bract, and possesses a finer texture. Towards its base on one side it is folded on itself and encloses the hop seed, if one has developed. This form of bract is called a "bracteole," and has developed from the small scale at the base of one of the ovaries of the flower (Fig. 32 *b*).

Remove all the Bracts from a Hop-Cone and Examine the Stem or "Strig" of the Hop.—The strig or main axis, which is thickly covered with fine short hairs, has a zigzag form and it will be observed that on the outer angle of each bend, and

FIG. 34.—Axis or Strig of Hop Cone.

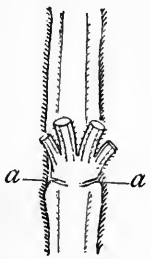


FIG. 35.—Group of Floral Axes on Hop Strig (much enlarged).

therefore placed alternately on the strig, are groups of short irregular branches (Fig. 34).

On examining a group of the branches with a pocket lens it will be found to consist of four

short axes radiating from a common point of attachment, and that the two central axes are rather longer than the side ones (Fig. 35). In the early flowering stage of the hop-cone each axis originally carried an ovary partly enclosed by a small scale or bracteole growing at its base (Fig. 32, *c* and *b*). After fertilisation the ovary developed into a seed, and the small scale-like bracteole situated at the base of the ovary developed into a full-sized bracteole enclosing the seed. So in a perfect hop-cone developed from a fertilised flower each of the four minor axes composing a group of branches on the hop strig carries a seed (or an abortive ovary), enclosed by a bracteole.

Now examine very carefully the strig of the hop immediately below the group of branches which bear the seeds and bracteoles. Two small scars will be observed to which small portions of leaf are frequently attached (see Fig. 35, *a a*). These are the points of attachment of two stipular bracts. The stipular bracts are in reality a pair of stipules belonging to a leaf which has not developed its blade. Occasionally, however, a monstrous development of the missing blade between the two stipules is observed and the hop-cone appears interspersed with small green leaves.

Now confirm the position of the two stipular bracts and the four bracteoles by examining a complete hop-cone, and note that the four short branches carrying the bracteoles and seeds proceed

from a point in the axil of the true leaf-bract represented by the space between the two stipular bracts.

The hop-cone is, therefore, composed of groups of two stipular bracts and four bracteoles placed alternately on opposite sides of the main axis or strig of the hop.

The Lupulin Glands of the Hop.—These glands, often called "lupulin," "condition" or "hop meal," are golden-yellow, pollen-like grains attached to various parts of the hop-cone. Botanically they are regarded as glandular hairs.

If the bracteoles of a hop-cone are examined with a pocket lens it will be observed that a large number of these glands are attached to their outer surface and are especially numerous near their base. The thin skin or perigonium, enclosing the seed, is also thickly covered with them. Some also are usually found on the inner surface of the stipular bracts.[1] On the number and size of these lupulin glands depends to a large extent the value of the hop, as they contain the essential oil and resins ("condition") which are of so much importance to the brewer.

Examine Lupulin Glands with the Microscope when Attached to a Bract.—It will be noticed that they are spherical, very thin-walled cells, enclosing an oily liquid. Their points of attachment to the

[1] See *Hops and their Botanical and Commercial Aspect*, by E. Gros, p. 23.

leaf are very slender and consequently they are readily broken off when touched.

Compare the Bared Strig of Different Varieties of Hops.—It will be noticed that the distance between the groups of branches is variable. A numerical expression of the variability may be obtained by counting the number of groups of branches in three-quarters of an inch of strig. The number found may vary from about five to about eight, and the expression "density number" is often applied to it, as it expresses the relative compactness of the bracts of the hop-cone. The density number of the hop-cone taken in conjunction with the shape of its bracts is of great use in helping to classify or identify the different varieties of hops.[1]

The student should now receive instruction in the commercial methods of judging and valuing hops. It is essential that this instruction should be given personally by a skilled instructor, who should be provided with a large number of samples of different kinds and qualities of hops for purposes of illustration. The student should recognise, however, that attainment of the knowledge necessary to form a sound judgment of the value and qualities of hops can only be gained by extensive experience.

[1] For much valuable information concerning the different varieties of hops, see a paper entitled "The Hop and its English varieties," by J. Percival, *Journal of the Royal Agricultural Society*, 1901, vol. lxii.

Chemical Examination of Hops.—The chemical examination of hops is usually confined to determinations of the resins and moisture, and to the detection of sulphur.

Determination of the Soft and Hard Resins.—The method is based on the consideration that both the soft and hard resins are soluble in ether, but that the soft resins alone are soluble in light petroleum spirit.[1]

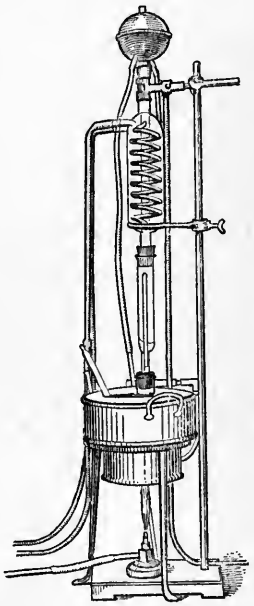


Fig. 36.—Apparatus for Extraction of Hop Resins.

Weigh accurately about 3 grms. of an average sample of the hops to be examined, place them in a cone of filter-paper, and transfer to a 100 c.c. Soxhlet extractor. Connect the apparatus by means of a well-fitting cork with a wide-mouthed flask containing about 100 c.c. of petroleum ether. The petroleum ether should possess a constant boiling point of about 125° F., and should be fractionally distilled, if necessary. The top of the Soxhlet extractor should be connected by means of a cork with an efficient form of reflux condenser (see Fig. 36).

The flask containing the petroleum ether is

[1] For full information concerning this method see *Laboratory Text-Book for Brewers*, by Lawrence Briant.

immersed in a water bath, which is kept at a temperature of about 155° F. During the extraction process the soft resins of the hop are only slowly removed by the petroleum ether, and the operation must be continued for about twenty-four hours. After extraction is complete, the flask is disconnected from the apparatus, and its contents filtered through a small paper filter into a small wide-mouthed tared flask. The petroleum ether is then distilled off, and the flask containing the soft resins placed in a water oven at a temperature of about 140° F. until its weight is constant. The gain in weight of the flask represents the amount of soft resins obtained.

After removal of the soft resins from the hops with petroleum ether, the hops are again extracted with ordinary ethylic ether for the purpose of removing the hard resins. About 100 c.c. of ether is used, and the temperature of the water bath surrounding the flask containing the ether should be kept at about 135° F. Extraction is complete in about twelve hours, when the ethereal extract is filtered as in the previous operation, and evaporation and drying also carried on as before.

The amounts of soft and hard resins found may be calculated as percentages on the hops used, or as percentages on the dried hops when the amount of moisture in the original hops is known.

Special precautions must be taken when conducting the above experiments to guard as far as

possible against the danger of explosions, as the vapour of the liquids employed is highly inflammable.

Determination of Moisture.—This can be done with a sufficient amount of accuracy for ordinary purposes by drying about 3 grms. of the hops in a water oven at 212° F. until the weight is constant. Most of the essential oil of the hops is driven off, but as the total amount is small the error involved is but slight.

Detection of Sulphur.—This method refers to the detection of free sulphur which may have gained access to the hops during the operation of treating the growing hops with finely divided sulphur for the prevention of mould.

Weigh out 5 grms. of hops and place them in a beaker with 250 c.c. distilled water. Add about 5 grms. of pure freshly slaked lime, and boil the mixture for twenty minutes. Filter a portion of the solution, cool and proceed to test it at once with a freshly prepared dilute solution of nitro-prusside of sodium. The test may be conducted by adding a few drops of the nitro-prusside solution to about 5 c.c. of the extract in a test-tube, or by bringing drops of the two solutions in conjunction on a white porcelain tile. The formation of a red colour indicates the presence of sulphur, due to the reaction of the nitro-prusside with hydro-sulphide of calcium formed by the action of calcium hydroxide on the free sulphur present in the hops.

The relative intensity of the colours found when comparing samples of hops indicates to some extent the relative amounts of sulphur present. When conducting this test the hop extract must be tested immediately after preparation, and it should be noted that the colour reaction with nitro-prusside is transient. As small quantities of sulphur are very generally found in English hops, the student should examine samples of Continental or Pacific hops for a negative reaction if required. These hops rarely show the presence of sulphur.

As a confirmatory test for the colour reaction, the student should add a few drops of sulphuric acid to a little of the hop decoction, and, after heating it, note the presence of sulphuretted hydrogen by its characteristic odour.

Estimation of Tannic Acid.—This estimation is rarely made. For information the student is referred to a paper by J. Heron on the tannin of hops (*Journal of the Federated Institutes of Brewing*, 1896, vol. ii., p. 162).

TABLE I.

Solution Factors for Carbo-hydrates at Various Densities. Specific Gravity at 15·5° C. (From Brown, Morris and Millar, *Journal of the Chemical Society*, 1897, vol. lxxi., p. 72.)

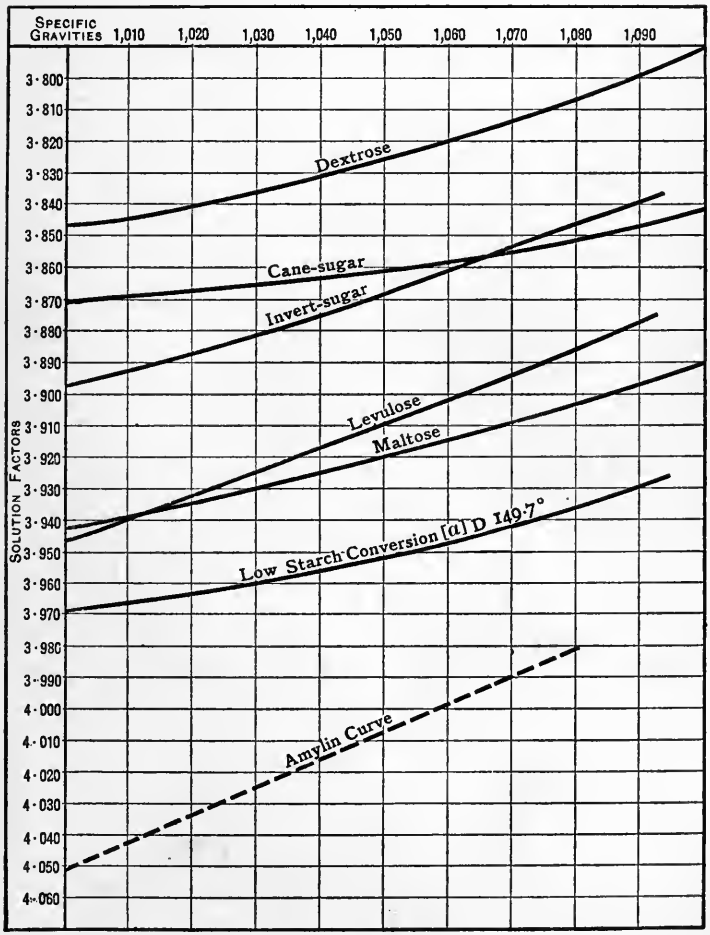


TABLE II.

Divisors for the Transformation Products of the Hydrolysis of Starch corresponding to $[\alpha]_D$ and R. (Brown, Morris and Millar, *Journal of the Chemical Society*, 1897, vol. lxxi., p. 84.)

$[\alpha]_D$	R.	Sp. Gr. 1010.	Sp. Gr. 1020.	Sp. Gr. 1030.	Sp. Gr. 1040.
198·8	5	4·038	4·031	4·023	4·015
195·6	10	4·033	4·027	4·020	4·013
192·4	15	4·029	4·023	4·017	4·010
189·2	20	4·024	4·019	4·013	4·008
186·0	25	4·019	4·014	4·010	4·005
182·8	30	4·014	4·011	4·006	4·001
179·6	35	4·009	4·005	4·002	3·997
176·4	40	4·004	4·001	3·997	3·993
173·2	45	3·999	3·996	3·992	3·989
170·0	50	3·994	3·991	3·987	3·985
166·8	55	3·989	3·986	3·983	3·979
163·6	60	3·984	3·980	3·977	3·974
160·4	65	3·979	3·975	3·972	3·969
157·2	70	3·973	3·970	3·966	3·963
154·0	75	3·968	3·964	3·961	3·957
150·8	80	3·962	3·958	3·955	3·951
147·6	85	3·957	3·952	3·948	3·945
144·4	90	3·951	3·946	3·942	3·938
141·2	95	3·945	3·940	3·935	3·931
138·0	100	3·939	3·934	3·929	3·924

TABLE III.

Reducing Values of Varying Quantities of Dextrose, Levulose and Invert-Sugar under Standard Conditions. (From Brown, Morris and Millar, *Journal of the Chemical Society*, 1897, vol. lxxi., p. 281.)

Dextrose.				Levulose.				Invert-Sugar.		
Dextrose. Grms.	Cu. Grms.	CuO. Grms.	Cu. Corresponding to 1 Grm. Dextrose.	Levulose. Grms.	Cu. Grms.	CuO. Grms.	Cu. Corresponding to 1 Grm. Levulose.	Invert-Sugar. Grms.	Cu. Grms.	CuO. Grms.
·050	·1030	·1289	2·060	·050	·0923	·1155	1·846	·050	·0975	·1221
·055	·1134	·1422	2·061	·055	·1027	·1287	1·858	·055	·1076	·1349
·060	·1238	·1552	2·063	·060	·1122	·1407	1·870	·060	·1176	·1474
·065	·1342	·1682	2·062	·065	·1216	·1524	1·871	·065	·1275	·1598
·070	·1443	·1809	2·061	·070	·1312	·1645	1·874	·070	·1373	·1721
·075	·1543	·1935	2·058	·075	·1405	·1761	1·873	·075	·1468	·1840
·080	·1644	·2061	2·055	·080	·1500	·1881	1·875	·080	·1566	·1963
·085	·1740	·2187	2·046	·085	·1590	·1993	1·871	·085	·1662	·2084
·090	·1834	·2299	2·038	·090	·1686	·2114	1·873	·090	·1755	·2200
·095	·1930	·2420	2·033	·095	·1774	·2224	1·868	·095	·1848	·2317
·100	·2027	·2538	2·027	·100	·1862	·2331	1·862	·100	·1941	·2430
·105	·2123	·2662	2·024	·105	·1952	·2447	1·859	·105	·2034	·2550
·110	·2218	·2781	2·020	·110	·2040	·2558	1·855	·110	·2128	·2668
·115	·2313	·2900	2·012	·115	·2129	·2669	1·851	·115	·2220	·2783
·120	·2404	·3014	2·003	·120	·2215	·2777	1·846	·120	·2311	·2898
·125	·2496	·3130	1·997	·125	·2303	·2887	1·843	·125	·2400	·3009
·130	·2585	·3241	1·990	·130	·2390	·2997	1·840	·130	·2489	·3121
·135	·2675	·3354	1·981	·135	·2477	·3106	1·834	·135	·2578	·3232
·140	·2762	·3463	1·973	·140	·2559	·3209	1·828	·140	·2663	·3339
·145	·2850	·3573	1·964	·145	·2641	·3311	1·822	·145	·2750	·3448
·150	·2934	·3673	1·956	·150	·2723	·3409	1·815	·150	·2832	·3546
·155	·3020	·3787	1·948	·155	·2805	·3517	1·811	·155	·2915	·3655
·160	·3103	·3891	1·940	·160	·2889	·3622	1·806	·160	·3002	·3764
·165	·3187	·3996	1·931	·165	·2972	·3726	1·803	·165	·3086	·3869
·170	·3268	·4098	1·922	·170	·3053	·3828	1·799	·170	·3167	·3971

TABLE III.—*continued.*

Dextrose.				Levulose.				Invert-Sugar.		
Dextrose. Grms.	Cu. Grms.	CuO. Grms.	Cu. Corresponding to 1 Grm. Dextrose.	Levulose. Grms.	Cu. Grms.	CuO. Grms.	Cu. Corresponding to 1 Grm. Levulose.	Invert-Sugar. Grms.	Cu. Grms.	CuO. Grms.
·175	·3350	·4200	1·914	·175	·3134	·3930	1·793	·175	·3251	·4076
·180	·3431	·4302	1·906	·180	·3216	·4032	1·787	·180	·3331	·4177
·185	·3508	.4399	1·896	·185	·3297	·4134	1·782	·185	·3410	·4276
·190	·3590	·4501	1·890	·190	·3377	·4234	1·777	·190	·3490	·4376
·195	·3668	·4599	1·881	·195	·3457	·4335	1·773	·195	·3570	·4476
·200	·3745	·4689	1·872	·200	·3539	·4431	1·769	·200	·3650	·4570
·205	·3822	·4792	1·863	·205	·3616	·4534	1·765	·205	·3726	·4672

The sugar values for weights of Cu or CuO lying between any of the weights given in the above table must be arrived at by calculation.

Example.—The amount of dextrose corresponding with ·2385 grm. Cu is required. On referring to the table ·2313 grm. Cu corresponds with ·115 grm. dextrose; and ·2404 grm. Cu with ·120 grm. dextrose. Hence ·2404 – ·2313 = ·0091 grm. Cu; and ·120 – ·115 = ·005 grm. dextrose. Therefore ·0091 grm. Cu = ·005 grms. dextrose in the portion of the table used. Now the difference between the amount of Cu found, ·2385, and the nearest lower amount in the table, ·2313 grm. is ·0072 grm. Hence: ·0091 : ·005 : : ·0072 : ·004. Therefore ·115 + ·004 = ·119 grm. dextrose corresponding to ·2385 grm. Cu.

TABLE IV.

Reducing Values of Varying Quantities of Maltose under Standard Conditions. (From Brown, Morris and Millar, *Journal of the Chemical Society*, 1897, vol. lxxi., p. 100.)

Maltose Grms.	Cu. Grms.	CuO. Grms.	Cu. Corresponding to 1 Grm. Maltose.	Maltose. Grms.	Cu. Grms.	CuO. Grms.	Cu. Corresponding to 1 Grm. Maltose.
·070	·0772	·0966	1·1029	·190	·2072	·2593	1·0953
·075	·0826	·1034	1·1026	·195	·2126	·2661	1·0949
·080	·0880	·1102	1·1023	·200	·2180	·2729	1·0946
·085	·0934	·1169	1·1020	·205	·2234	·2797	1·0943
·090	·0988	·1237	1·1017	·210	·2288	·2865	1·0940
·095	·1042	·1305	1·1013	·215	·2342	·2933	1·0937
·100	·1097	·1373	1·1010	·220	·2397	·3000	1·0933
·105	·1151	·1441	1·1007	·225	·2451	·3068	1·0930
·110	·1205	·1509	1·1004	·230	·2505	·3136	1·0927
·115	·1259	·1576	1·1001	·235	·2559	·3203	1·0924
·120	·1313	·1644	1·0997	·240	·2613	·3272	1·0921
·125	·1367	·1712	1·0994	·245	·2667	·3340	1·0917
·130	·1422	·1779	1·0991	·250	·2722	·3407	1·0914
·135	·1476	·1848	1·0988	·255	·2776	·3475	1·0911
·140	·1530	·1916	1·0985	·260	·2830	·3543	1·0908
·145	·1584	·1983	1·0981	·265	·2884	·3610	1·0905
·150	·1634	·2051	1·0978	·270	·2938	·3678	1·0901
·155	·1692	·2119	1·0975	·275	·2992	·3747	1·0898
·160	·1747	·2186	1·0972	·280	·3047	·3814	1·0895
·165	·1801	·2254	1·0969	·285	·3101	·3882	1·0892
·170	·1855	·2323	1·0965	·290	·3155	·3950	1·0889
·175	·1909	·2490	1·0962	·295	·3209	·4017	1·0885
·180	·1963	·2458	1·0959	·300	·3264	·4085	1·0882
·185	·2017	·2526	1·0956	·305	·3318	·4154	1·0879

TABLE V.

Spirit Indication Table showing Degrees of Gravity lost in Malt Worts during Fermentation. Distillation Process. (Graham, Hoffmann, and Redwood.)

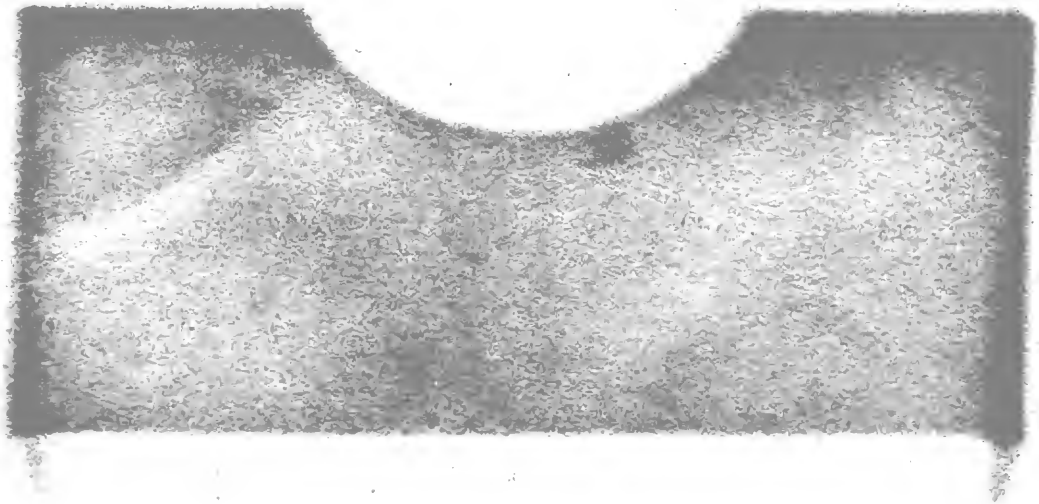
Degrees of Spirit Indication.	·0	·1	·2	·3	·4	·5	·6	·7	·8	·9
0	—	·3	·6	·9	1·2	1·5	1·8	2·1	2·4	2·7
1	3·0	3·3	3·7	4·1	4·4	4·8	5·1	5·5	5·9	6·2
2	6·6	7·0	7·4	7·8	8·2	8·6	9·0	9·4	9·8	10·2
3	10·7	11·1	11·5	12·0	12·4	12·9	13·3	13·8	14·2	14·7
4	15·1	15·5	16·0	16·4	16·8	17·3	17·7	18·2	18·6	19·1
5	19·5	19·9	20·4	20·9	21·3	21·8	22·2	22·7	23·1	23·6
6	24·1	24·6	25·0	25·5	26·0	26·4	26·9	27·4	27·8	28·3
7	28·8	29·2	29·7	30·2	30·7	31·2	31·7	32·2	32·7	33·2
8	33·7	34·3	34·8	35·4	35·9	36·5	37·0	37·5	38·0	39·6
9	39·1	39·7	40·2	40·7	41·2	41·7	42·2	42·7	43·2	43·7
10	44·2	44·7	45·1	45·6	46·0	46·5	47·0	47·5	48·0	48·5
11	49·0	49·6	50·1	50·6	51·2	51·7	52·2	52·7	53·3	53·8
12	54·3	54·9	55·4	55·9	56·4	56·9	57·4	57·9	58·4	58·9
13	59·4	60·0	60·5	61·1	61·6	62·2	62·7	63·3	63·8	64·3
14	64·8	65·4	65·9	66·5	67·1	67·6	68·2	68·7	69·3	69·9
15	70·5	71·1	71·7	72·3	72·9	73·5	74·1	74·7	75·3	75·9

TABLE VI.

Original Gravity Determination. Table for Ascertaining the Correction for Acid.

Excess Per Cent. of Acetic Acid in the Beer.	Corresponding Degrees of Spirit Indication.									
	·00	·01	·02	·03	·04	·05	·06	·07	·08	·09
·0	—	·02	·04	·06	·07	·08	·09	·11	·12	·13
·1	·14	·15	·17	·18	·19	·21	·22	·23	·24	·26
·2	·27	·28	·29	·31	·32	·33	·34	·35	·37	·38
·3	·39	·40	·42	·43	·44	·46	·47	·48	·49	·51
·4	·52	·53	·55	·56	·57	·59	·60	·61	·62	·64
·5	·65	·66	·67	·69	·70	·71	·72	·73	·75	·76
·6	·77	·78	·80	·81	·82	·84	·85	·86	·87	·89
·7	·90	·91	·93	·94	·95	·97	·98	·99	1·00	1·02
·8	1·03	1·04	1·05	1·07	1·08	1·09	1·10	1·11	1·13	1·14
·9	1·15	1·16	1·18	1·19	1·21	1·22	1·23	1·25	1·26	1·28
1·0	1·29	1·31	1·33	1·35	1·36	1·37	1·38	1·40	1·41	1·42

THE ABERDEEN UNIVERSITY PRESS LIMITED.



TC.
Bro.

162300

Author Brown, Adrian J.

Title Laboratory studies for brewing students

University of Toronto
Library

DO NOT
REMOVE
THE
CARD
FROM
THIS
POCKET

M Loughran
Donald McKay

Acme Library Card Pocket
Under Pat. "Ref. Index File"
Made by LIBRARY BUREAU